Exhibit A

1: Biochem Biophys Res Commun. 1997 Oct 29;239(3):794-8.

Related Articles, Links

ELSEVIER
FULL-TEXT ARTICLE

Alternative splicing of the primary Fas transcript generating soluble Fas antagonists is suppressed in the failing human ventricular myocardium.

Schumann H, Morawietz H, Hakim K, Zerkowski HR, Eschenhagen T, Holtz J, Darmer D.

Institute of Pathophysiology, Martin Luther University Halle-Wittenberg, Germany.

Apoptosis of cardiomyocytes has been proposed as a factor contributing to severe heart failure. Since the trigger for apoptotic cellular suicide in nonischemic myocardium is unknown, we analyzed in human myocardial tissue the expression of the apoptosis-inducing membrane receptor Fas/APO-1 and of its alternatively spliced soluble isoforms which antagonize Fas by binding of the Fas ligand. Using reverse transcription polymerase chain reaction (RT-PCR) we found mRNA for Fas and 5 isoforms in nonfailing left ventricles, whereas Fas and only one isoform (FasExo6Del) were detectable in failing left ventricles. Standard calibrated, competitive RT-PCR revealed no significant increase of Fas mRNA in failing compared to nonfailing ventricles. However, the mRNA for FasExo6Del, expressed nearly on the same level as Fas in nonfailing ventricles, was decreased about 3-fold in failing ventricles. We propose that this altered expression of the Fas system renders the myocardium more susceptible for Fas-mediated apoptosis in end-stage heart failure.

PMID: 9367848 [PubMed - indexed for MEDLINE]

1: Biochem Biophys Res Commun. 1997 Aug 28;237(3):516-20. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Four mutant alleles of the insulin receptor gene associated with genetic syndromes of extreme insulin resistance.

Kadowaki H, Takahashi Y, Ando A, Momomura K, Kaburagi Y, Quin JD, MacCuish AC, Koda N, Fukushima Y, Taylor SI, Akanuma Y, Yazaki Y, Kadowaki T.

Institute for Diabetes Care and Research, Asahi Life Foundation, Tokyo, Japan.

We identified four novel mutant alleles of the insulin receptor gene in three patients with genetic syndromes associated with insulin resistance. Two mutant alleles of the insulin receptor gene were identified in a patient with the Rabson-Mendenhall syndrome who was a compound heterozygote for a mutation at the 3'-splice acceptor site of intron 4 (AG-->GG), the first mutation causing an aberrant splicing at this locus, and a deletion of eight base pairs in exon 12. The second patient with leprechaunism was also a compound heterozygote for a deletion of one base pair in exon 19 and a mutation, Thr910-->Met, which causes impaired receptor processing. Interestingly, the third patient with type A syndrome was a simple heterozygote for the identical one base pair deletion. The fact that the same one base pair deletion links to type A in a simple heterozygote and to leprechaunism in a compound heterozygote appears consistent with the hypothesis that the severity of mutations will determine the phenotype.

Publication Types:
- Case Reports

PMID: 9299395 [PubMed - indexed for MEDLINE]

1: Hum Mutat. 1997;10(3):201-6.

Related Articles, Links

Constitutive APC exon 14 skipping in early-onset familial adenomatous polyposis reveals a dramatic quantitative distortion of APC gene-specific isoforms.

Bala S, Sulekova Z, Ballhausen WG.

Institut fuer Humangenetik der Universitaet, Erlangen, Germany.

Adenomatous polyposis coli (APC) gene transcripts skipping exon 14 in combination with the alternatively spliced exons 9 and 10A contribute to the heterogeneity of physiological APC mRNA isoforms. Here we report on a novel genotype-phenotype correlation in familial adenomatous polyposis (FAP) with early onset of disease and malignancy due to an APC exon 14 splice defect. Compared to controls, two affected individuals of a FAP kindred presented with a significantly distorted APC mRNA isoform pattern in B lymphocytes. As a result of an A-->G transition in the canonical AG-splice acceptor dinucleotide of exon 14, expression levels of all APC mRNA isoforms without exon 14 were dramatically increased and those with exon 14 were simultaneously decreased. Skipping of exon 14 is a physiological event also seen in nonmalignant cells, which results in a frameshift to produce low-molecular-weight APC proteins. Western blot analysis of the patients' lymphoblastoid B cells revealed the identification of intracellularly stable APC protein isoforms with an Mr of 55-67 kDa and, thus, the first demonstration of APC proteins encoded by exon 14-skipped transcripts. We postulate that the quantitatively imbalanced expression of these physiological APC light chains represents a novel pathogenetic mechanism associated with predisposition to FAP.

PMID: 9298819 [PubMed - indexed for MEDLINE]

1: Am J Med Genet. 1997 Oct 3;72(1):94-105. Related Articles, Links

Ehlers-Danlos syndrome type VIIA and VIIB result from splice-junction mutations or genomic deletions that involve exon 6 in the COL1A1 and COL1A2 genes of type I collagen.

Byers PH, Duvic M, Atkinson M, Robinow M, Smith LT, Krane SM, Greally MT, Ludman M, Matalon R, Pauker S, Quanbeck D, Schwarze U.

Department of Pathology, University of Washington, Seattle 98195-7470, USA.
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Ehlers-Danlos syndrome (EDS) type VII results from defects in the conversion of type I procollagen to collagen as a consequence of mutations in the substrate that alter the protease cleavage site (EDS type VIIA and VIIB) or in the protease itself (EDS type VIIC). We identified seven additional families in which EDS type VII is either dominantly inherited (one family with EDS type VIIB) or due to new dominant mutations (one family with EDS type VIIA and five families with EDS type VIIB). In six families, the mutations alter the consensus splice junctions, and, in the seventh family, the exon is deleted entirely. The COL1A1 mutation produced the most severe phenotypic effects, whereas those in the COL1A2 gene, regardless of the location or effect, produced congenital hip dislocation and other joint instability that was sometimes very marked. Fractures are seen in some people with EDS type VII, consistent with alterations in mineral deposition on collagen fibrils in bony tissues. These new findings expand the array of mutations known to cause EDS type VII and provide insight into genotype/phenotype relationships in these genes.

PMID: 9295084 [PubMed - indexed for MEDLINE]

1: Int J Cancer. 1997 Aug 22;74(4):443-5. Related Articles, Links

Soluble CD44 splice variants in metastasizing human breast cancer.

Martin S, Jansen F, Bokelmann J, Kolb H.

Diabetes Research Institute at the Heinrich-Heine University, Dusseldorf, Germany.

The local expression of CD44 splice variants in human breast cancer tissue has been previously shown to be associated with metastasis. We show here that elevated systemic serum levels of CD44 splice variants occur in breast cancer and may represent a new tool for staging and differential diagnosis. Sera of node-negative and node-positive breast cancer patients in comparison with healthy control subjects were analyzed for serum CD44 (sCD44) and 2 different splice variants (v5 and v6). Node-positive breast cancer patients showed significantly ($p < 0.01$) elevated levels of sCD44-v5 and -v6 splice variants in comparison to node-negative patients and healthy controls. None of the node-negative breast cancer patients or healthy controls showed elevated levels of both sCD44-v5 and -v6. Interestingly, no differences were seen for serum levels of non-spliced sCD44-standard between the 3 groups. Soluble forms of CD44 variants may promote migration of tumor cells. This may occur through interference with tumor cell adhesion or by modulation of immune defense mechanisms.

PMID: 9291436 [PubMed - indexed for MEDLINE]

1: Am J Med Genet. 1997 Sep 5;71(4):378-83. Related Articles, Links

The ornithine transcarbamylase (OTC) gene: mutations in 50 Japanese families with OTC deficiency.

Matsuda I, Tanase S.

Department of Pediatrics, Kumamoto University School of Medicine, Japan.

Mutations in the OTC gene in 50 Japanese families with OTC deficiency were reviewed in relation to the phenotype of the patients and predicted structure of the mutant enzyme. Similar to other X-linked diseases, mutant alleles in OTC deficiency are highly heterogeneous. Mutations observed in male patients with neonatal onset of the disease included base insertion/deletion, exon skipping, and nonsense and missense mutations in exon 4, 5, 6, or 7. OTC activity was essentially undetectable in this group of patients. These mutations possibly resulted in unstable mRNA or truncated protein, or involved the active site or core domain of the enzyme leading to structural changes. In male patients with late onset, abnormalities observed were missense mutations in exons 2, 4, 8, 9, and 10, and missense mutations plus donor site errors involving exons 4, 5, and 6. OTC activity in these patients was 8.1 +/- 6.3% of the control and most mutations occurred on the surface of the protein. In female patients, age at onset ranged from 19 months to 7 years, depending on residual OTC activities (4.5 to 33% of the control). Most mutations in this group were similar to those seen in male patients with neonatal onset, i.e., nonsense and missense mutations in exons 5 and 6, and exon skipping, leading to null enzyme activity. These collective data can serve for genetic counseling and monitoring in prenatal care.

Publication Types:
- Review
- Review, Tutorial

PMID: 9286441 [PubMed - indexed for MEDLINE]

1: Am J Med Genet. 1997 Aug 22;71(3):366-70. Related Articles, Links

Mutation producing alternative splicing of exon 26 in the COL1A2 gene causes type IV osteogenesis imperfecta with intrafamilial clinical variability.

Zolezzi F, Valli M, Clementi M, Mammi I, Cetta G, Pignatti PF, Mottes M.

Institute of Biology and Genetics, University of Verona, Italy.

We have characterized a familial form of osteogenesis imperfecta (OI). Following the identification by ultrasound of short limbs and multiple fractures in a fetus at 25 weeks of gestation, the family was referred with a provisional diagnosis of severe OI. We detected subtle clinical and radiological signs of OI in the father and in the paternal grandmother of the proposita, who had never received a diagnosis of OI. Linkage analysis indicated COL1A2 as the disease locus. Heteroduplex analysis of reverse transcription-polymerase chain reaction (RT-PCR) amplification products of pro alpha2(I) mRNA from an affected member and subsequent sequencing of the candidate region demonstrated the presence of normal transcripts and a minority of transcripts lacking exon 26 (54 bp) of COL1A2. Sequencing of PCR-amplified genomic DNA identified an A --> G transition in the moderately conserved +3 position of the IVS 26 donor splice site. The mutant pre-mRNA molecules were alternatively spliced, yielding both full-length and deleted transcripts that represented less than 30% of the total pro alpha2(I) mRNA. The biochemical data on type I collagen synthesized by dermal fibroblasts showed intracellular retention of the mutant protein; failure to detect the shortened alpha2(I) chains either in the medium or in the cell layer may be the consequence of their instability at physiological temperature. These observations justified the mild resulting phenotype.

Publication Types:
- Case Reports

PMID: 9268111 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1998 Feb 27;273(9):5013-9. Related Articles, Links

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Relative increase in Alzheimer's disease of soluble forms of cerebral Abeta amyloid protein precursor containing the Kunitz protease inhibitory domain.

Moir RD, Lynch T, Bush AI, Whyte S, Henry A, Portbury S, Multhaup G, Small DH, Tanzi RE, Beyreuther K, Masters CL.

Department of Pathology, The University of Melbourne, Parkville, 3052, Australia and The Mental Health Research Institute of Victoria, Parkville 3052, Australia.

Although a number of studies have examined amyloid precursor protein (APP) mRNA levels in Alzheimer's disease (AD), no clear consensus has emerged as to whether the levels of transcripts for isoforms containing a Kunitz protease inhibitory (KPI)-encoded region are increased or decreased in AD. Here we compare AD and control brain for the relative amounts of APP protein containing KPI to APP protein lacking this domain. APP protein was purified from the soluble subcellular fraction and Triton X-100 membrane pellet extract of one hemisphere of AD ($n = 10$), normal ($n = 7$), and neurological control ($n = 5$) brains. The amount of KPI-containing APP in the purified protein samples was determined using two independent assay methods. The first assay exploited the inhibitory action of KPI-containing APP on trypsin. The second assay employed reflectance analysis of Western blots. The proportion of KPI-containing forms of APP in the soluble subcellular fraction of AD brains is significantly elevated ($p < 0.01$) compared with controls. Species containing a KPI domain comprise 32-41 and 76-77% of purified soluble APP from control and AD brains, respectively. For purified membrane-associated APP, 72-77 and 65-82% of control and AD samples, respectively, contain a KPI domain. Since KPI-containing species of APP may be more amyloidogenic (Ho, L., Fukuchi, K., and Yonkin, S. G. (1996) J. Biol. Chem. 271, 30929-30934), our findings support an imbalance of isoforms as one possible mechanism for amyloid deposition in sporadic AD.

PMID: 9478949 [PubMed - indexed for MEDLINE]

1: Thyroid. 1998 Jan;8(1):43-7. Related Articles, Links

Serum concentration of soluble Fas in patients with autoimmune thyroid diseases.

Shimaoka Y, Hidaka Y, Okumura M, Takeoka K, Tada H, Amino N.

Department of Laboratory Medicine, Osaka University Medical School, Suita, Japan.

Fas is an apoptosis-signaling receptor molecule found on the surface of a number of cell types. Malfunction of the Fas system accelerates autoimmune diseases, whereas its exacerbation may cause tissue destruction. Soluble Fas (sFas) molecule lacks the transmembrane domain due to alternative splicing and blocks Fas-mediated apoptosis. This study investigated serum levels of sFas in autoimmune thyroid diseases. Serum levels of sFas were determined by enzyme-linked immunosorbent assay in 46 patients with Graves' disease, 32 patients with Hashimoto's thyroiditis, 14 patients with silent thyroiditis, and 24 normal controls. Compared with normal subjects (1.43+/-0.37 ng/mL), sFas was increased in thyrotoxic patients with Graves' disease (1.89+/-0.47 ng/mL, $p < 0.001$), and was decreased in patients with Graves' disease in remission (1.02+/-0.41 ng/mL, $p < 0.001$) and in euthyroid patients with Hashimoto's thyroiditis (0.97+/-0.25 ng/mL, $p < 0.0001$), but was normal in hypothyroid patients with Hashimoto's thyroiditis and in thyrotoxic patients with silent thyroiditis. Thus, changes in serum levels of sFas could not be explained by changes in serum thyroid hormones, although sFas concentration correlated with free thyroxine ($r = 0.692$, $p < 0.0001$). Also, the levels of sFas significantly correlated with the activities of TSH receptor antibody in Graves' disease ($r = 0.671$, $p < 0.0001$). Increased sFas in Graves' disease suggests increased expression of alternatively spliced Fas mRNA variant that produces sFas protein and decreased of cell surface expression of Fas, and may induce thyroid cell growth and production of TSH receptor antibody by protecting against apoptosis of thyroid cells and autoreactive B cells. Decreased sFas in Hashimoto's thyroiditis suggests decreased Fas mRNA variant and increased full-length Fas mRNA and membrane Fas, and may induce destruction of thyroid cells by promoting apoptosis of thyroid cells.

PMID: 9492152 [PubMed - indexed for MEDLINE]

1: Jpn J Cancer Res. 1998 Jan;89(1):47-52. Related Articles, Links

Japanese Cancer Association Cancer Science

Relatively high expression ratio of sex hormone-binding globulin exon VII splicing variant to wild-type mRNA in human uterine cervical cancers.

Misao R, Nakanishi Y, Fujimoto J, Tamaya T.

Department of Obstetrics and Gynecology, Gifu University School of Medicine.

We have demonstrated the intracellular expression of sex hormone-binding globulin (SHBG) exon VII splicing variant mRNA in human uterine cervical cancer using reverse transcription-polymerase chain reaction-Southern blot and DNA sequencing analyses. Analysis of the missing base pairs proved they corresponded to the entire exon VII, which is considered to encode a portion of the steroid-binding site, suggesting that the steroid-binding affinity of the variant protein might be different from that of the wild-type SHBG. In uterine cervical cancers, the wild-type mRNA levels were lower ($P<0.01$) and the ratio of the SHBG variant to wild-type mRNA levels was higher ($P<0.01$) than in the normal cervix. In cervical adenocarcinomas, the wild-type mRNA levels were higher ($P<0.05$) and the ratio of the SHBG variant to wild-type mRNA levels was lower ($P<0.05$) than in cervical keratinizing squamous cell carcinomas. There was no difference in expression among the clinical stages of cervical cancers. These results suggest that a relative increase of intracellular variant SHBG protein in human uterine cervical cancers might be involved in the disruption of the normal estrogen dependence.

PMID: 9510475 [PubMed - indexed for MEDLINE]

1: Cancer Lett. 1998 Feb 27;124(2):143-8. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Splicing patterns of type XI collagen transcripts act as molecular markers for osteochondrogenic tumors.

Matsui Y, Kimura T, Tsumaki N, Nakata K, Yasui N, Araki N, Hashimoto N, Uchida A, Ochi T.

Department of Orthopaedic Surgery, Osaka University Medical School, Suita, Japan.

Primary transcripts for three distinct alpha chains of the type XI collagen molecule (alpha1(XI), alpha2(XI) and alpha3[XI]) undergo tissue-specific alternative splicing during the process of osteochondrogenesis. In the present study, we analyzed the splicing patterns of type XI collagen genes in osteochondrogenic tumors as well as in various normal tissues using the reverse transcription-polymerase chain reaction method. Analysis of normal subjects revealed the coordinated expression of short alpha1(XI), alpha2(XI) and alpha3(XI) transcripts in the normal differentiated cartilage. Osteochondroma followed this pattern, reflecting the highly chondrogenic phenotype of this benign tumor. Another benign tumor, chondroblastoma, exclusively expressed the long alpha1(XI) transcript, probably reflecting the lack of a chondrogenic nature. Among malignant chondrogenic tumors, the splicing patterns of type XI collagen transcripts were more complex, showing dissociated expression of long alpha1(XI) and short alpha2(XI) mRNAs. This expression pattern may reflect heterogeneous cell populations and may also reflect various levels of cell differentiation in malignant tumors. In addition, short alpha3(XI) expression switched to the long transcript as chondrosarcomas became more aggressive. Thus, the alternative splicing of type XI collagen genes seems to be oncodevelopmentally regulated and splicing analysis may therefore be a useful marker for chondrogenic tumors.

PMID: 9500203 [PubMed - indexed for MEDLINE]

1: J Clin Invest. 1998 Feb 1;101(3):588-94.

Related Articles, Links

Two aberrant splicings caused by mutations in the insulin receptor gene in cultured lymphocytes from a patient with Rabson-Mendenhall's syndrome.

Takahashi Y, Kadowaki H, Ando A, Quin JD, MacCuish AC, Yazaki Y, Akanuma Y, Kadowaki T.

Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113-0033, Japan.

Rabson-Mendenhall's syndrome is one of the most severe forms of insulin resistance syndrome. We analyzed an English patient described elsewhere and found novel mutations in both alleles of the insulin receptor gene. One is a substitution of G for A at the 3' splice acceptor site of intron 4, and the other is an eight-base pair deletion in exon 12. Both decrease mRNA expression in a cis-dominant manner, and are predicted to produce severely truncated proteins. Surprisingly, nearly normal insulin receptor levels were expressed in the patient's lymphocytes, although the level of expression assessed by immunoblot was approximately 10% of the control cells. Insulin binding affinity was markedly reduced, but insulin-dependent tyrosine kinase activity was present. Analyzing the insulin receptor mRNA of the patient's lymphocytes by reverse transcription PCR, we discovered aberrant splicing caused by activation of a cryptic splice site in exon 5, resulting in a four-amino acid deletion and one amino acid substitution, but restoring an open reading frame. Skipped exon 5, another aberrant splicing, was found in both the patient and the mother who had the heterozygotic mutation, whereas activation of the cryptic splice site occurred almost exclusively in the patient. Transfectional analysis in COS cells revealed that the mutant receptor produced by cryptic site activation has the same characteristics as those expressed in patient's lymphocytes. We speculate that this mutant receptor may be involved in the relatively long survival of the patient by rescuing otherwise more severe phenotypes resulting from the complete lack of functional insulin receptors.

PMID: 9449692 [PubMed - indexed for MEDLINE]

1: Hum Genet. 1998 Jan;102(1):15-20. Related Articles, Links

Characterization of MLH1 and MSH2 alternative splicing and its relevance to molecular testing of colorectal cancer susceptibility.

Genuardi M, Viel A, Bonora D, Capozzi E, Bellacosa A, Leonardi F, Valle R, Ventura A, Pedroni M, Boiocchi M, Neri G.

Istituto di Genetica Medica, Universita Cattolica del Sacro Cuore, Rome, Italy.

The phenomenon of alternative splicing in the DNA mismatch repair genes MLH1 and MSH2 was extensively investigated by coupled reverse transcription-polymerase chain reaction in different human tissues, including 42 mononuclear blood cell samples--31 obtained from familial colon cancer patients or their at-risk relatives and 11 from healthy blood donors--7 normal colonic mucosae, 4 established human cancer cell lines, 8 colorectal tumors, and one sample each of ileum, liver, muscle, thymus, breast, and EBV-transformed lymphoblasts. Several isoforms were observed for each gene. Products of MLH1 alternative splicing included mRNAs lacking alternative exons 6/9, 9, 9/10, 9/10/11, 10/11, 12, 16, and 17. For MSH2, products lacking exons 5, 13, 2 through 7, and 2 through 8 were identified. The levels of expression were found to vary among different samples. All isoforms were found in a relevant fraction (43-100%) of the mononuclear blood cell samples, as well as in other tissues. The splicing variants were also detected in normal colonic mucosa, with the exceptions of the MLH1 -6/9 and -10/11 and the MSH2 -13 isoforms. Germline mutations of MLH1 and MSH2 confer constitutional predisposition to the development of colorectal cancer and other neoplasms. A substantial proportion of the mutations identified so far involve alterations of the normal splicing process. Knowledge of the existence of multiple alternative splicing events, not caused by genomic DNA changes, is important for the evaluation of the results of molecular diagnostic tests based on RNA analysis.

PMID: 9490293 [PubMed - indexed for MEDLINE]

1: Cancer Res. 1998 Feb 15;58(4):609-13. Related Articles, Links

Short alternative splice transcripts of the mdm2 oncogene correlate to malignancy in human astrocytic neoplasms.

Matsumoto R, Tada M, Nozaki M, Zhang CL, Sawamura Y, Abe H.

Laboratory for Molecular Brain Research, Hokkaido University School of Medicine, Sapporo, Japan.

The mdm2 oncogene encodes a 90-kDa nuclear phosphoprotein that binds and inhibits the function of the p53 tumor suppressor protein. It was recently reported that the expression of alternatively spliced variants of mdm2 correlated with malignancy in ovarian tumors and bladder carcinomas. We analyzed the presence of alternatively spliced mdm2 variants and studied their correlation to p53 status in a total of 66 human astrocytic tumors, including 32 glioblastomas multiforme, 17 anaplastic astrocytomas, 12 astrocytomas, and 5 pilocytic astrocytomas, using a specific nested reverse transcription-PCR technique. The full-length mdm2 transcript was demonstrated in all of the cases. Multiple-sized PCR products were found in 29 cases. Two of 5 pilocytic astrocytomas (40%), none of 12 astrocytomas, and 5 of 17 anaplastic astrocytomas (29%) showed alternative splice variants. In contrast, 22 of 32 glioblastomas (69%) showed the presence of splice variants, demonstrating a significantly higher frequency than in lower-grade astrocytomas ($P < 0.0003$). A majority of the splice variants were 707 base-type (mdm2-b), which was confirmed by sequence analysis. There was no apparent correlation of the presence of mdm2 splice variants with p53 gene status. These results suggest a new role for mdm2, independent of p53 gene status, as an oncogene in the development of malignant astrocytic tumors.

PMID: 9485008 [PubMed - indexed for MEDLINE]

1: J Med Genet. 1998 Jan;35(1):45-8. Related Articles, Links

Do intronic mutations affecting splicing of WT1 exon 9 cause Frasier syndrome?

Kikuchi H, Takata A, Akasaka Y, Fukuzawa R, Yoneyama H, Kurosawa Y, Honda M, Kamiyama Y, Hata J.

Department of Pathology, Keio University School of Medicine, Tokyo, Japan.

The WT1 gene, one of the genes responsible for Wilms tumour, is thought to play a crucial role in the development of the kidneys and gonads. This gene encodes four protein isoforms resulting from two alternative splicing sites, one of which involves inclusion or exclusion of lysine, threonine, and serine (KTS) between the third and fourth zinc finger domains. WT1 is virtually always mutationally inactivated in patients with Denys-Drash syndrome. We analysed WT1 in eight patients who had been diagnosed as having this syndrome, and identified five previously unknown mutations affecting splicing donor sites of intron 9. These mutations affect alternative splicing. The isoforms retaining KTS are not produced. The clinical features of the patients with these intronic mutations were consistent with those of Frasier syndrome, characterised by a more slowly progressive nephropathy than Denys-Drash syndrome, associated streak gonads, and no Wilms tumour development. Our results indicate that WT1 isoforms, including/excluding KTS, have different functions in tumorigenesis and organogenesis of the kidneys and gonads.

PMID: 9475094 [PubMed - indexed for MEDLINE]

1: Cancer Lett. 1998 Jan 9;122(1-2):187-93. Related Articles, Links

DPC4 splice variants in neuroblastoma.

Kageyama H, Seki N, Yamada S, Sakiyama S, Nakagawara A.

Division of Biochemistry, Chiba Cancer Center Research Institute, Japan.

One of the loci for neuroblastoma suppressor genes is chromosome 18q21 where the DPC4 tumor suppressor gene, as well as the DCC and MADR2 genes, is located. DPC4 is a molecule of the TGF-beta signal which regulates differentiation of the neural crest precursor cells from which neuroblastoma originates. During the search for the significance of DPC4 as a candidate neuroblastoma suppressor gene, we found that there are at least two variant forms of the DPC4 transcripts by using the reverse-transcriptase-PCR procedure. The subsequent sequencing analysis has revealed that one is missing exons 5 and 6 and the other is missing exons 4-6. Both splice variants were frequently observed in neuroblastomas and at low levels in normal tissues. Though the functional role of the DPC4 splice variants is unknown, they might be important in regulating the TGF-beta signaling not only in neuroblastomas but also in other tumors and normal tissues.

PMID: 9464509 [PubMed - indexed for MEDLINE]

1: Acta Paediatr Jpn. 1997 Dec;39(6):685-9. Related Articles, Links

Early cardiac failure in a child with Becker muscular dystrophy is due to an abnormally low amount of dystrophin transcript lacking exon 13.

Ishigaki C, Patria SY, Nishio H, Yoshioka A, Matsuo M.

Division of Genetics, International Center for Medical Research, Kobe, Japan.

Two Japanese brothers with Becker muscular dystrophy were shown by polymerase chain reaction (PCR) and cDNA sequence analysis to produce a dystrophin gene transcript lacking a single exon: that is, number 13. Despite having the same deletion mutation, the brothers showed clearly different clinical phenotypes: the younger brother developed cardiac failure at the age of nine, while the elder brother was asymptomatic. As alternative splicing was not responsible for this clinical difference, the amount of dystrophin transcript was examined by using reverse transcription semi-nested and parallel PCR. The results showed that the amount of the dystrophin transcript in the younger brother was 20% of that of the elder brother. This finding suggested that lesser amount of dystrophin transcript in the younger brother was responsible for the early onset of cardiac failure. This would represent a novel molecular mechanism for dystrophinopathy.

Publication Types:
- Case Reports

PMID: 9447758 [PubMed - indexed for MEDLINE]

1: Cancer Res. 1997 Dec 15;57(24):5579-83. Related Articles, Links

Expression of sex hormone-binding globulin exon VII splicing variant messenger RNA in human uterine endometrial cancers.

Misao R, Nakanishi Y, Fujimoto J, Tamaya T.

Department of Obstetrics and Gynecology, Gifu University School of Medicine, Japan.

We have demonstrated the intracellular expression of sex hormone-binding globulin (SHBG) exon VII splicing variant mRNA in human uterine endometrial cancer using the reverse transcription-PCR-Southern blot and DNA sequencing analyses. Analysis of the missing base pairs proved that they corresponded to the entire exon VII, which is considered to encode a portion of the steroid-binding site, suggesting that the steroid-binding affinity of this variant might be different from that of the SHBG wild type. In uterine endometrial cancers, the wild-type mRNA levels significantly ($P < 0.01$) decreased, and the ratio of the SHBG variant to wild-type mRNA levels ($P < 0.01$) increased with the advance of histological dedifferentiation. These results suggest that dedifferentiation of endometrial cancers might induce a reduction in their estrogen-dependent properties via intracellular SHBG.

PMID: 9407970 [PubMed - indexed for MEDLINE]

1: Int J Oncol. 1998 Mar;12(3):525-33. Related Articles, Links

Expression of CD44 splicing isoforms in lung cancers: dominant expression of CD44v8-10 in non-small cell lung carcinomas.

Sasaki JI, Tanabe KK, Takahashi K, Okamoto I, Fujimoto H, Matsumoto M, Suga M, Ando M, Saya H.

Department of Tumor Genetics, Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto 860, Japan.

We examined CD44 isoform expression in 138 frozen tissue samples, which included primary lung carcinomas, adjacent non-tumorous lung tissues and benign lung diseases, by both reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemical analyses. CD44v8-10 mRNA and protein were dominantly expressed in non-small cell lung carcinomas (NSCLC), while non-tumorous tissues principally expressed CD44s and small cell lung carcinomas (SCLC) expressed either CD44s or no detectable CD44. These results indicate that CD44v8-10 is the dominant splicing isoform in NSCLC and can be practically utilized as a diagnostic marker and therapeutical target in NSCLC.

1: Am J Hum Genet. 1998 Feb;62(2):269-77. Related Articles, Links

Nearby stop codons in exons of the neurofibromatosis type 1 gene are disparate splice effectors.

Hoffmeyer S, Nurnberg P, Ritter H, Fahsold R, Leistner W, Kaufmann D, Krone W.

Abteilung Humangenetik, Universitat Ulm, Ulm, Germany. sven.hoffmeyer@medizin.uni-ulm.de

Stop mutations are known to disrupt gene function in different ways. They both give rise to truncated polypeptides because of the premature-termination codons (PTCs) and frequently affect the metabolism of the corresponding mRNAs. The analysis of neurofibromin transcripts from different neurofibromatosis type 1 (NF1) patients revealed the skipping of exons containing PTCs. The phenomenon of exon skipping induced by nonsense mutations has been described for other disease genes, including the CFTR (cystic fibrosis transmembrance conductance regulator) gene and the fibrillin gene. We characterized several stop mutations localized within a few base pairs in exons 7 and 37 and noticed complete skipping of either exon in some cases. Because skipping of exon 7 and of exon 37 does not lead to a frameshift, PTCs are avoided in that way. Nuclear-scanning mechanisms for PTCs have been postulated to trigger the removal of the affected exons from the transcript. However, other stop mutations that we found in either NF1 exon did not lead to a skip, although they were localized within the same region. Calculations of minimum-free-energy structures of the respective regions suggest that both changes in the secondary structure of the mRNA and creation or disruption of exonic sequences relevant for the splicing process might in fact cause these different splice phenomena observed in the NF1 gene.

PMID: 9463322 [PubMed - indexed for MEDLINE]

1: Oncol Rep. 1998 Jan-Feb;5(1):31-4. Related Articles, Links

Evaluation of the 5' spliced form of human cathepsin B mRNA in colorectal mucosa and tumors.

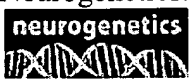
Hizel C, Ferrara M, Cure H, Pezet D, Dechelotte P, Chipponi J, Rio P, Bignon YJ, Bernard-Gallon D.

Laboratoire d'Oncologie Moleculaire, INSERM CRI 9402, Centre Jean Perrin, 58 rue Montalembert, B.P. 392, 63011 Clermont-Ferrand Cedex 1, France.

To evaluate the 5' spliced form of human cathepsin B mRNA in colorectal mucosa and tumors, we have determined the ratio of the spliced form for the exon 2/the complete form of cathepsin B mRNAs obtained by RT-PCR. Such ratio is significantly higher in colorectal tumors than in colorectal mucosa ($p < 0.05$, Kruskal-Wallis test) or in skeletal muscle ($p < 0.05$). Moreover, 2-fold more complete form than the spliced mRNA was found in the tumors than in the colorectal mucosa. Our data indicate that the alternative splicing of human cathepsin B mRNA in the 5'UTR may be considered as an indicator of the cellular transformation, in colorectal cancer.

PMID: 9458288 [PubMed - indexed for MEDLINE]

1: Neurogenetics. 1997 May;1(1):65-71. Related Articles, Links



Exon 5 encoded domain is not required for the toxic function of mutant SOD1 but essential for the dismutase activity: identification and characterization of two new SOD1 mutations associated with familial amyotrophic lateral sclerosis.

Zu JS, Deng HX, Lo TP, Mitsumoto H, Ahmed MS, Hung WY, Cai ZJ, Tainer JA, Siddique T.

Department of Neurology, Northwestern Medical School, Chicago, IL 60611-3008, USA.

Two new mutations in the gene encoding cytoplasmic Cu,Zn superoxide dismutase (SOD1) have been discovered in patients with familial amyotrophic lateral sclerosis (FALS). These mutations result in the truncation of most of the polypeptide segment encoded by exon 5, one by the formation of a stop codon in codon 126 (L126Z) and the other by inducing alternative splicing in the mRNA (splicing junction mutation). These two mutants of SOD1 result in a FALS phenotype similar to that observed in patients with missense mutations in the SOD1 gene, establishing that exon 5 is not required for the novel toxic functions of mutant SOD1 associated with ALS. These mutant enzymes are present at very low levels in FALS patients, suggesting elevated toxicity compared to mutant enzymes with single site substitutions. This increased toxicity likely arises from the extreme structural and functional changes in the active site channel, beta-barrel fold, and dimer interface observed in the mutant enzymes, including the loss of native dismutase activity. In particular, the truncation of the polypeptide chain dramatically opens the active site channel, resulting in a marked increase in the accessibility and flexibility of the metal ions and side chain ligands of the enzyme active site. These structural changes are proposed to cause a decrease in substrate specificity and an increase in the catalysis of harmful chemical reactions such as peroxidation.

Publication Types:
- Case Reports

PMID: 10735277 [PubMed - indexed for MEDLINE]

1: Endocr Pathol. 1995 Summer;6(2):91-101. Related Articles, Links

Expression of the Neural Cell Adhesion Molecule NCAM by Peptide- and Steroid-Producing Endocrine Cells and Tumors: Alternatively Spliced Forms and Polysialylation.

Lahr G, Mayerhofer A.

The adhesive properties of neural cell adhesion molecules (NCAMs) can be modified by alternative splicing of the primary transcript or by posttranslational modifications, such as sialylation. In this article, we describe distinct forms of alternative splicing and posttranslational modification of the extracellular domain of NCAM of various endocrine tissues and derived tumor cells of the rat and of steroid- and peptide-hormone producing endocrine cells in humans. NCAM-140 is the major isoform expressed in the rat adrenal gland, adenohypophysis, and in granulosa and granulosa-lutein cells. NCAM-180 is predominant in the neurohypophysis. Polysialylated NCAM is expressed in different endocrine tissues and tumor cells of the rat. Different amounts of NCAM mRNA containing the "extra-exon" VASE at the exon 7/8 splice boundary were detected in endocrine cells of rats. Human granulosa cells in culture undergo luteinization. During this process, the VASE-containing NCAM isoform is supplemented by an alternatively spliced isoform without this insert. Thus, modifications of NCAM may be important for adhesive interactions in normal and neoplastic endocrine cells. In addition, the differential expression and the alternative splicing of NCAM during luteinization of granulosa cells raise the possibility that NCAM could be involved in folliculogenesis and the formation of the corpus luteum in humans.

PMID: 12114645 [PubMed - as supplied by publisher]

1: Breast Cancer. 1997 Jul 31;4(2):57-66. Related Articles, Links

Alterations and Polymorphisms of the Estrogen Receptor Gene in Breast Cancer.

Iwase H, Kobayashi S.

Department of Surgery II, Nagoya City University Medical School, 1 Kawasumi, Mizuho-ku, Nagoya 467, Japan.

The existence of hormone-independent tumors is a substantial problem for the present endocrine treatment of breast cancers. Recently, numerous variant estrogen receptors (ERs) at the mRNA level have been detected with base pair insertions, transitions, and deletions, as well as alternative splicing, yielding deletion of exon 3, 5, or 7. It has been shown that the loss of hormone dependence in breast tumors is partly due to the presence of mutated or truncated ERs that can activate the transcription of an estrogen-regulatable gene in the absence of estrogen. The mechanism of the loss of hormone dependency is, however, still very complex. Thus, further work assessing the correlation between clinical behavior and ER variants is required to determine whether these variants play a role in hormone-resistant disease. Additionally, a possible linkage to the ER gene has been found in some breast cancer families, suggesting that either the ER gene itself or an adjacent gene may be breast cancer susceptibility genes.

PMID: 11091579 [PubMed - as supplied by publisher]

1: Clin Cancer Res. 1996 Aug;2(8):1251-4. Related Articles, Links

Novel CD44 messenger RNA isoforms in human thyroid and breast tissues feature unusual sequence rearrangements.

Ermak G, Jennings T, Boguniewicz A, Figge J.

Departments of Medicine and Pathology, Albany Medical College, Albany, New York 12208, USA.

CD44 is a family of cell surface proteins implicated in adhesion interactions and tumor metastasis. Multiple CD44 mRNA isoforms arise from alternative splicing of variant exons (termed v1-v10). We recently discovered a novel CD44 mRNA isoform in human papillary thyroid cancers featuring a junction between subsegments of exons 4 and 13 (v8). The sequence ACAG was repeated at both the donor and acceptor sites in the genomic DNA (G. Ermak et al., Cancer Res., 56: 1037-1042, 1996). We used reverse transcription-PCR to characterize expression of this isoform in a panel of thyroid lesions. In addition, we assayed three cryopreserved human breast cancers and two samples of normal breast tissue (from female subjects who had undergone cosmetic mammoplasty) to determine whether a similar isoform is present in breast carcinomas. Levels of the novel isoform were up-regulated in 88% of the goiters, adenomas, and papillary cancers, but were undetectable in cases of thyroiditis and absent or low-level in four samples of normal thyroid tissue. The three breast cancers each yielded a 546-bp PCR product that was not detected in normal breast tissue. The PCR product from one of the breast cancers was cloned, and sequence analysis revealed a novel mRNA isoform featuring a junction between exon 3 and an internal site within exon 13 (v8). The sequence GCTTCAG was repeated at both the donor and acceptor sites in the genomic DNA. These results show that human thyroid and breast tissues contain novel CD44 mRNA isoforms featuring unusual rearrangements at repeated sequences. Further studies are warranted to determine whether the expression of this class of isoforms correlates with growth status.

PMID: 9816294 [PubMed - indexed for MEDLINE]

1: Cytogenet Cell Genet. 1998;81(3-4):183-8. Related Articles, Links

KARGER Full Text

FHIT gene transcript alterations occur frequently in myeloproliferative and myelodysplastic diseases.

Luan X, Ramesh KH, Cannizzaro LA.

Albert Einstein College of Medicine, Montefiore Medical Center, Department of Pathology, Bronx, NY, USA.

Twenty-five primary biopsy samples, obtained from patients diagnosed with chronic/acute myeloproliferative disorders, myelodysplastic disorders, in addition to seven cell lines established from patients with leukemias arrested at different stages of myeloid differentiation, were examined for alterations in an alternatively spliced form of the FHIT gene. Transcript alterations of this gene were detected in 80% of the primary biopsies and in two of the cell lines. Reverse transcription PCR (RT-PCR) detected deletions of one or more specific exons in the translated or untranslated portion of the FHIT gene. Point mutations in exons 3, 4, and 5 of the FHIT gene were also detected in 4 patients by single stranded conformational PCR analysis. Transcript alterations were detected in all primary hematopoietic samples which contained chromosome abnormalities, as well as, in hematopoietic disorders which did not show alterations of the 3p14 region. These findings suggest FHIT gene involvement in the transformation of hematopoietic stem cells to leukemia.

PMID: 9730598 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 1996 Apr 2;93(7):2884-8.

Aberrant platelet-derived growth factor alpha-receptor transcript as a diagnostic marker for early human germ cell tumors of the adult testis.

Mosselman S, Looijenga LH, Gillis AJ, van Rooijen MA, Kraft HJ, van Zoelen EJ, Oosterhuis JW.

Department of Cell Biology, University of Nijmegen, the Netherlands.

Testicular germ cell tumors are the most common form of cancer in young adult males. They result from a derangement of primordial germ cells, and they grow out from a noninvasive carcinoma-in-situ precursor. Since carcinoma in situ can readily be cured by low-dose irradiation, there is a great incentive for non- or minimally invasive methods for detection of carcinoma in situ. We have recently shown that human Tera-2 embryonal carcinoma cells, obtained from a nonseminomatous testicular germ cell tumor, show alternative splicing and alternative promoter use of the platelet-derived growth factor alpha-receptor gene, giving rise to a unique 1.5-kb transcript. In this study we have set up a reverse transcriptase-polymerase chain reaction strategy for characterization of the various transcripts for this receptor. Using this technique, we show that a panel of 18 seminomas and II nonseminomatous testicular germ cell tumors all express the 1.5-kb transcript. In addition, a panel of 27 samples of testis parenchyma with established carcinoma in situ were all found to be positive for the 1.5-kb transcript, while parenchyma lacking carcinoma in situ, placenta, and control semen were all negative. These data show that the 1.5-kb platelet-derived growth factor alpha-receptor transcript can be used as a highly selective marker for detection of early stages of human testicular germ cell tumors.

PMID: 8610136 [PubMed - indexed for MEDLINE]

1: Oncogene. 1996 May 16;12(10):2187-92. Related Articles, Links

Identification of a novel interleukin-15 (IL-15) transcript isoform generated by alternative splicing in human small cell lung cancer cell lines.

Meazza R, Verdiani S, Biassoni R, Coppolecchia M, Gaggero A, Orengo AM, Colombo MP, Azzarone B, Ferrini S.

Instituto Nazionale per la Ricerca sul Cancro, Genova Italy.

IL-15 is a cytokine promoting growth and differentiation of T, B and NK lymphocytes. By RT-PCR analysis, using primers allowing amplification of the entire IL-15 mRNA coding region, 9/11 small cell lung cancer (SCLC) cell lines displayed detectable IL-15 gene expression. In addition to the expected band sizing 524 bp, a larger band was also observed. Cloning and sequence analysis of the larger cDNA from two SCLC cell lines revealed a size of 643 hp due to the presence of additional 119 hp within the previously reported IL-15 cDNA sequence. The 119 hp sequence matched with an IL-15 genomic sequence downstream the IL-15 second coding exon and may represent a previously unreported alternative exon (exon A). The SCLC-associated IL-15 mRNA isoform has a shorter open reading frame (ORF) due to stop codons in exon A, followed by a new AUG codon. The predicted IL-15 precursor protein displays a shorter signal peptide but shares the same aminoacidic composition of mature IL-15 protein. A possible functional role of IL-15, different from 'IL-2-like' activity, in human tumours, is suggested.

PMID: 8668345 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1996 May 24;271(21):12603-9.

Alternative splicing of the human cholesteryl ester transfer protein gene in transgenic mice. Exon exclusion modulates gene expression in response to dietary or developmental change.

Yang TP, Agellon LB, Walsh A, Breslow JL, Tall AR.

Department of Medicine, Columbia University, New York, New York 10032, USA.

The plasma cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl ester from high density lipoprotein to other lipoproteins. The human DETP gene produces two forms of mRNA, with or without exon 9 (E9)-derived sequences. To study the function and regulation of alternative splicing the CETP gene, transgenic mice were prepared 1) with the metallothionein (mT) promoter driving an E9-deleted construct (mT.CETP(-E9) transgene), and 2) with the natural flanking regions (NFR) controlling expression of genomic sequences which permit alternative splicing of E9 (NFR.CETP(+/-E9) transgene). With zinc induction, the mT.CETP(-E9) transgene gave rise to abundant E9-deleted CETP mRNA in liver and small intestine, but only relatively small amounts of E9-deleted protein were found in plasma. The E9-deleted form of CETP was inactive in lipid transfer and produced no changes in plasma lipoprotein profile. The NFR.CETP(+/-E9) transgene gave rise to full-length (FL) and E9-deleted forms of CETP mRNA in liver and spleen. In response to hypercholesterolemia induced by diet and breeding into an apoE gene knock-out background, the FL CETP mRNA was induced more than the E9-deleted mRNA, resulting in a 2-fold increase in ratio of FL/E9-deleted mRNA. The expression of CETP mRNA was found to be developmentally regulated. In NFR.CETP(+/-E9) transgenic mice CETP mRNA levels were markedly increased in the liver and small intestine in the perinatal period and decreased in adult mice, whereas CETP mRNA in the spleen was low in perinatal mice and increased in adults. The developmental increase in CETP mRNA in the liver and spleen was preceded by an increased ratio of FL/E9-deleted forms. Thus, the E9-deleted mRNA appears to be poorly translated and/or secreted, and the cognate protein is inactive in lipid transfer and lipoprotein metabolism. CETP gene expression was found to be highly regulated in a tissue-specific fashion during development. Increased CETP gene expression during development or in response to hypercholesterolemia is associated with preferential accumulation of the full-length CETP mRNA.

PMID: 8647871 [PubMed - indexed for MEDLINE]

1: Neuroreport. 1996 Jul 8;7(10):1680-4. Related Articles, Links

Structure and alternative splicing of the presenilin-2 gene.

Prihar G, Fuldner RA, Perez-Tur J, Lincoln S, Duff K, Crook R, Hardy J, Philips CA, Venter C, Talbot C, Clark RF, Goate A, Li J, Potter H, Karran E, Roberts GW, Hutton M, Adams MD.

Suncoast Alzheimer's Disease Laboratories, Department of Psychiatry, University of South Florida, Tampa 33613, USA.

Missense mutations in the presenilin-1 (PS-1) and presenilin-2 (PS-2) genes have been shown to be causes of autosomal dominant Alzheimer's disease (the AD3 and AD4 loci, respectively). Alternative splicing has previously been reported in the PS-1 gene. In this study, elucidation of intron/exon boundary sequences revealed that PS-2 is encoded by 10 coding exons. In addition, PS-2 cDNA cloning and RT-PCR using RNA from a variety of normal tissues revealed the presence of alternatively spliced products. These products included species with in frame omissions of exon 8 and simultaneous omissions of exons 3 and 4.

PMID: 8904781 [PubMed - indexed for MEDLINE]

1: Cell Calcium. 1996 Jul;20(1):63-72. Related Articles, Links

The calcium-binding protein calretinin-22k, an alternative splicing product of the calretinin gene is expressed in several colon adeno carcinoma cell lines.

Gander JC, Bustos-Castillo M, Stuber D, Hunziker W, Celio M, Schwaller B.

Institute of Histology and General Embryology, University of Fribourg, Switzerland.

An alternatively spliced mRNA for the calcium-binding protein calretinin (CR) is present in the colon adenocarcinoma cell line WiDr. As a consequence of a frame shift, the resulting protein, calretinin-22k (CR-22k), consists of the first 178 amino acids of calretinin followed by a carboxy-terminal peptide of 14 amino acids that is not present in full-length calretinin. Antibodies specific for this C-terminal region have been generated by 2 different methods. A peptide corresponding to the specific C-terminal region of CR-22k was either chemically synthesized and coupled to a carrier protein or was expressed in Escherichia coli as a carboxyterminal fusion to a carrier protein applying recombinant techniques. Both antisera produced in rabbits were tested in Western blots and immuno-histochemical experiments. The antisera recognized human recombinant CR-22k overexpressed in E. coli, but not fulllength calretinin and stained fixed WiDr cells. The presence of CR-22k was also confirmed in the colon cell lines CO115/3 in which mRNA coding for CR-22k mRNA coding for CR-22k mRNA is present as well as in the lines COLO205 and LS-180, all of which also express full-length calretinin. Although the intracellular distribution of CR-22k and CR are similar as evidenced by immunohistochemical stainings, CR-22k is preferentially localized in the nucleus in the cell lines LS-180 and Co115/3 suggesting potentially different roles for the two proteins.

PMID: 8864572 [PubMed - indexed for MEDLINE]

1: Proc Assoc Am Physicians. 1996 Jul;108(4):308-14. Related Articles, Links

A case of Becker muscular dystrophy resulting from the skipping of four contiguous exons (71-74) of the dystrophin gene during mRNA maturation.

Patria SY, Alimsardjono H, Nishio H, Takeshima Y, Nakamura H, Matsuo M.

International Center for Medical Research, Kobe University School of Medicine, Japan.

The mutations in one-third of both Duchenne and Becker muscular dystrophy patients remain unknown because they do not involve gross rearrangements of the dystrophin gene. Here we report the first example of multiple exon skipping during the splicing of dystrophin mRNA precursor encoded by an apparently normal dystrophin gene. A 9-year-old Japanese boy exhibiting excessive fatigue and high serum creatine kinase activity was examined for dystrophinopathy. An immunohistochemical study of muscle tissue biopsy disclosed faint and discontinuous staining of the N-terminal and rod domains of dystrophin but no staining at all of the C-terminal domain of dystrophin. The dystrophin transcript from muscle tissue was analyzed by the reverse transcriptase polymerase chain reaction. An amplified product encompassing exons 67-79 of dystrophin cDNA was found to be smaller than that of the wild-type product. Sequence analysis of this fragment showed that the 3' end of exon 70 was directly connected to the 5' end of exon 75 and, thus, that exons 71-74 were completely absent. As a result, a truncated dystrophin protein lacking 110 amino acids from the C-terminal domain should result from translation of this truncated mRNA, and the patient was diagnosed as having Becker muscular dystrophy at the molecular level. Genomic DNA was analyzed to identify the cause of the disappearance of these exons. Every exon-encompassing region could be amplified from genomic DNA, indicating that the dystrophin gene is intact. Furthermore, sequencing of these amplified products did not disclose any particular nucleotide change that could be responsible for the multiple exon skipping observed. Considering that exons 71-74 are spliced out alternatively in some tissue-specific isoforms, to suppose that the alternative splicing machinery is present in the muscle tissue of the index case and that it is activated by an undetermined mechanism is reasonable. These results illustrate a novel genetic anomaly that results in dystrophinopathy.

Publication Types:
- Case Reports

PMID: 8863344 [PubMed - indexed for MEDLINE]

1: Nucleic Acids Res. 1996 Jun 15;24(12):2347-51.

Exon skipping induced by cold stress in a potato invertase gene transcript.

Bournay AS, Hedley PE, Maddison A, Waugh R, Machray GC.

Department of Cell and Molecular Genetics, Scottish Crop Research Institute, Invergowrie,Dundee, UK.

We show that two invertase genes in potato, like most other plant invertase genes, include a very short second exon of 9 bp which encodes the central three amino acids of a motif highly conserved in invertases of diverse origin. This mini-exon is one of the smallest known in plants and pre-mRNA from these genes may be susceptible to alternative splicing, because of a potential requirement for specialized interaction with the splicing machinery to ensure correct processing for the production of a mature mRNA. No evidence of aberrant post-transcriptional processing was observed during normal invertase gene expression in potato. The fidelity of post-transcriptional processing of the pre-mRNA from one of the genes was perturbed by cold stress, resulting in the deletion of the mini-exon from some transcripts. This alternative splicing event occurred under cold stress in both leaf and stem, but was not induced by wounding. This adds an example of exon skipping and the induction of alternative processing by cold stress to the small number of transcripts which have been shown to exhibit alternative splicing in plants. The differential sensitivity of post-transcriptional processing to cold stress observed for the two transcripts examined will permit further dissection of the nucleotide sequence requirements for their accurate splicing.

PMID: 8710506 [PubMed - indexed for MEDLINE]

1: Biochim Biophys Acta. 1996 Jun 3;1307(1):83-8. Related Articles, Links

Modification of the alternative splicing process of testosterone-repressed prostate message-2 (TRPM-2) gene by protein synthesis inhibitors and heat shock treatment.

Kimura K, Yamamoto M.

Department of Biochemistry, National Defense Medical College, Saitama, Japan.

During the course of the study to examine the effect of cycloheximide on apoptosis-related genes, the variant rat testosterone-repressed prostate message-2 (TRPM-2) mRNA deficient of the exon 5 was found. The putative protein encoded by the variant TRPM-2 mRNA is only constituted from the N-terminal one-third portion of the ordinary TRPM-2 protein. The expression of the variant form was increased dramatically by cycloheximide treatment, while that of the ordinary form was not affected very much. The similar phenomenon was also observed by the use of other types of protein synthesis inhibitors, anisomycin and emetine. The enhancement of expression of the variant was observed in the rat treated with heat shock as well. The variant form was presumably generated by the exon skip mechanism. Systematic analyses of cycloheximide effect on the alternative splicing at various splicing junctions were performed. However, cycloheximide did not exhibit any remarkable effects on other types of alternative splicing, including exon skip in beta A4-amyloid protein precursor (APP) gene, alternative donor selection in Fas antigen gene and alternative acceptor selection in catechol O-methyltransferase (COMT) gene. These results indicated that the induction of exon skip by both protein synthesis inhibition and heat shock treatment occurs in a limited number of genes, if not only in TRPM-2.

PMID: 8652672 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab. 1996 Jun;81(6):2344-9.

Related Articles, Links

Presence of alternatively spliced transcripts of aromatase gene in human breast cancer.

Utsumi T, Harada N, Maruta M, Takagi Y.

Department of Surgery, School of Medicine, Fujita Health University, Aichi, Japan.

The expression of aromatase (estrogen synthetase) is tissue specifically regulated through the alternative use of multiple exons 1 and promotors. We have determined the amounts of aromatase messenger ribonucleic acid (mRNA) and which type of multiple exons 1 of the human aromatase gene is used in breast tissues of 49 patients with breast cancer by reverse transcription-PCR analysis. The aromatase mRNA levels in these breast cancer tissues (4.53 +/- 0.66 x 10(-3) attomoles/micrograms RNA) were significantly ($P < 0.01$) higher than those in 16 nonmalignant breast tissues (1.73 +/- 0.40 x 10(-3) amol/micrograms RNA). Aromatase mRNA in all nonmalignant breast tissue were transcribed from skin fibroblast/fetal liver-specific exon 1 (exon 1b) of the gene. In 23 breast cancer tissues, the utilization of multiple exons 1 in the aromatase mRNA was the same as that in nonmalignant breast tissues, whereas in the other 26 cases, it changed from exon 1b to ovary-specific exon 1 (exon 1c). Such switching of tissue-specific exons 1 may affect strict regulation of the tissue-specific expression of aromatase, leading to abnormal expression of the aromatase. The consequent overproduction of local estrogen might promote carcinogenesis or the proliferation of breast cancers.

PMID: 8964875 [PubMed - indexed for MEDLINE]

1: J Lipid Res. 1996 Jun;37(6):1213-23. Related Articles, Links

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A novel A-->G mutation in intron I of the hepatic lipase gene leads to alternative splicing resulting in enzyme deficiency.

Brand K, Dugi KA, Brunzell JD, Nevin DN, Santamarina-Fojo S.

Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA.

We have identified the underlying molecular defect in a patient with hepatic lipase (HL) deficiency presenting with hypertriglyceridemia and premature cardiovascular disease. DNA sequencing of polymerase chain reaction (PCR) amplified DNA and digestion with BsrI established homozygosity for an A-->G mutation in intron I of the patient's hepatic lipase gene. This mutation introduces an additional AG motif within a potential branch lariat signal located 13 bp upstream of the native 3' splice site. Two minigene constructs (normal and mutant) consisting of exons 1 and 2 as well as 192 bp of intron I of HL were generated by the overlap PCR extension method and transfected in human 293 cells. Sequence analysis of reverse transcribed, amplified cDNA generated from total RNA isolated from transfected cells demonstrated the presence of abnormally spliced products containing 13 and 78 additional bases as well as the accumulation of unspliced mRNA. No normally spliced mRNA was identified. Thus, the A-->G mutation disrupts normal splicing of intron I and generates a new AG site that is utilized as an alternative 3' splice signal leading to the most prominent RT-PCR product in vitro. Translation of these alternatively spliced products leads to premature termination resulting in the synthesis of a truncated, non-functional enzyme. The absence of normal HL protein in post heparin plasma of this patient was confirmed by Western blotting. DNA restriction analysis demonstrated that all four of the proband's children, who exhibit HL activity levels between those of the HL-deficient father and the mother with normal HL activity, are heterozygotes for the splice site mutation. Thus, our studies establish the functional significance of a novel mutation in the HL gene of a patient presenting with HL deficiency.

Publication Types:
- Case Reports

PMID: 8808756 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1996 May 31;271(22):13208-14.

Related Articles, Links

Alzheimer's disease betaA4 protein release and amyloid precursor protein sorting are regulated by alternative splicing.

Hartmann T, Bergsdorf C, Sandbrink R, Tienari PJ, Multhaup G, Ida N, Bieger S, Dyrks T, Weidemann A, Masters CL, Beyreuther K.

Zentrum fur Molekulare Biologie, University of Heidelberg, D-69120 Heidelberg, Federal Republic of Germany.

We show here that alternative splicing influences the polarized secretion of amyloid precursor protein (APP) as well as the release of its proteolytic 3-4-kDa fragments betaA4 and p3. In Madin-Darby canine kidney II cells stably transfected with various APP isoforms and APP mutants, APPsec was consistently secreted basolaterally. In contrast, Madin-Darby canine kidney II cells transfected with L-APP677, which occurs naturally by alternative splicing of exon 15, secreted this isoform both apically and basolaterally, while maintaining the basolateral sorting of endogenous APPsec. This suggests that the alternative splicing of APP exon 15 modulates the polarized sorting of secretory APP. The same alternative splicing event also decreased the production of betaA4 relative to p3. This is the first example of alternative splicing regulating polarized trafficking of a secretory protein.

PMID: 8662794 [PubMed - indexed for MEDLINE]

1: Nat Med. 1996 Aug;2(8):912-7. Related Articles, Links

Alternatively spliced mdm2 transcripts with loss of p53 binding domain sequences: transforming ability and frequent detection in human cancer.

Sigalas I, Calvert AH, Anderson JJ, Neal DE, Lunec J.

Cancer Research Unit, The Medical School, University of Newcastle upon Tyne, Framlington Place, UK.

The mdm2 oncogene encodes a 90-kilodalton nuclear phosphoprotein that binds and inactivates the p53 tumor suppressor protein. Here we report the observation of five alternatively spliced mdm2 gene transcripts in a range of human cancers and their absence in normal tissues. Transfection of NIH 3T3 cells with each of these forms gave foci of morphologically transformed cells. A higher frequency of splice variants lacking p53 binding domain sequences was found in late-stage and high-grade ovarian and bladder carcinomas. Four of the splice variants show loss of p53 binding, consistent with partial deletion of sequences encoding the p53 binding domain, but retain carboxyterminal zinc-finger domains. These observations suggest a reassessment of the transforming mechanisms of mdm2 and its relation to p53.

PMID: 8705862 [PubMed - indexed for MEDLINE]

1: Circulation. 1996 Aug 1;94(3):472-6.

Related Articles, Links

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Cardiac troponin T isoform expression correlates with pathophysiological descriptors in patients who underwent corrective surgery for congenital heart disease.

Saba Z, Nassar R, Ungerleider RM, Oakeley AE, Anderson PA.

Department of Pediatrics, Duke University, Durham, NC 27710, USA.

BACKGROUND: This study examined cardiac troponin T (cTnT) isoform expression in patients who had undergone surgery at Duke University Medical Center (Durham, NC) between December 1, 1993, and January 31, 1995, to correct congenital heart defects. The human heart expresses four cTnT isoforms (cTnT1 through cTnT4) whose sequence differences result from combinatorial alternative splicing of two exons. We have previously shown that cTnT4 is expressed at higher levels in severely failing hearts from transplant patients. In this study, we tested the hypothesis that congenital heart defects that have a more negative effect on myocardial function increase cTnT4 expression. We used the presence or absence of drug treatment for heart failure or congested circulation before surgery and the duration of inotropic support after corrective surgery as indicators of the pathophysiological state of the heart just before surgery. METHODS AND RESULTS: Right atrial appendage tissue was collected from 34 patients, 6 days to 35 years old (median age, 3.4 months). The amounts of the cTnT1 through cTnT4 isoforms, measured as a percentage of total cTnT, were determined from Western blots probed with MAb13-11, a cTnT-specific monoclonal antibody. We found that cTnT4 expression correlated positively with the duration of inotropic support and was higher in patients who received drug treatment before surgery than in those who did not. Furthermore, we found that the percent of cTnT4 was significantly higher in hearts with congenital defects that caused congestive failure than in hearts with tetralogy of Fallot. CONCLUSIONS: These findings suggest that in patients with congenital cardiac defects, cTnT4 expression is modulated by heart failure and is increased in hearts that are more hemodynamically stressed.

PMID: 8759091 [PubMed - indexed for MEDLINE]

1: J Lipid Res. 1996 Aug;37(8):1761-5. Related Articles, Links

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A new mutation in the gene for lysosomal acid lipase leads to Wolman disease in an African kindred.

Ries S, Aslanidis C, Fehringer P, Carel JC, Gendrel D, Schmitz G.

Institute of Clinical Chemistry and Laboratory Medicine, University of Regensburg, Germany.

Cholesteryl ester storage disease (CESD) and Wolman disease (WD) are both autosomal recessive disorders associated with reduced activity and genetic defects of lysosomal acid lipase (LAL). The strikingly more severe course of WD is caused by genetic defects of LAL that leave no residual enzymatic activity. Mutations at the exon 8/intron 8 transition of the LAL gene have been identified in several CESD and WD patients and are responsible for the manifestation of the disease. We have determined the genetic defect in a 3-month-old boy of African origin affected by WD. No enzymatic activity of the lysosomal acid lipase was detectable in white blood cells and cultured fibroblasts. Analysis of his LAL cDNA and genomic DNA revealed that he was homozygous for a mutation at position -3 of the exon 8 splice donor site. A C-->T transition leads to a nonsense codon and to a premature termination of the LAL protein at amino acid 277. Due to this mutation, a shorter LAL mRNA species was also generated that lacked exon 8 and was deficient of the nonsense codon. As a consequence, the protein synthesis proceeded to the natural termination codon, but the enzyme generated had an internal deletion of 24 amino acids (254-277) and was also inactive. These findings, together with our previous observations when analyzing the mutations in WD and CESD patients lead to the conclusion that the more severe WD is due to mutations that absolutely abolish lysosomal acid lipase (LAL) enzyme activity and the cholesteryl ester storage disease phenotype is due to mutations that allow some residual LAL activity to be manifested.

PMID: 8864960 [PubMed - indexed for MEDLINE]

1: J Clin Invest. 1996 Jul 15;98(2):358-64.

Related Articles, Links

An intronic mutation in a lariat branchpoint sequence is a direct cause of an inherited human disorder (fish-eye disease).

Kuivenhoven JA, Weibusch H, Pritchard PH, Funke H, Benne R, Assmann G, Kastelein JJ.

Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, The Netherlands.

The first step in the splicing of an intron from nuclear precursors of mRNA results in the formation of a lariat structure. A distinct intronic nucleotide sequence, known as the branchpoint region, plays a central role in this process. We here describe a point mutation in such a sequence. Three sisters were shown to suffer from fish-eye disease (FED), a disorder which is caused by mutations in the gene coding for lecithin:cholesterol acyltransferase (LCAT). Sequencing of the LCAT gene of all three probands revealed compound heterozygosity for a missense mutation in exon 4 which is reported to underlie the FED phenotype, and a point mutation located in intron 4 (IVS4:T-22C). By performing in vitro expression of LCAT minigenes and reverse transcriptase PCR on mRNA isolated from leukocytes of the patient, this gene defect was shown to cause a null allele as the result of complete intron retention. In conclusion, we demonstrated that a point mutation in a lariat branchpoint consensus sequence causes a null allele in a patient with FED. In addition, our finding illustrates the importance of this sequence for normal human mRNA processing. Finally, this report provides a widely applicable strategy which ensures fast and effective screening for intronic defects that underlie differential gene expression.

PMID: 8755645 [PubMed - indexed for MEDLINE]

1: Int J Cancer. 1997 Aug 7;72(4):574-80. Related Articles, Links

Identification of an exon 3 deletion splice variant androgen receptor mRNA in human breast cancer.

Zhu X, Daffada AA, Chan CM, Dowsett M.

Academic Department of Biochemistry, Royal Marsden Hospital, London, UK.

Androgens and androgen receptor (AR) are involved in many regulatory processes in the growth of female breast cells. Mutations in the AR gene and/or alterations of the AR protein sequence may be related to the development and progression of breast cancer. Using reverse transcription-polymerase chain reaction we have examined 31 female breast-cancer samples, 5 normal female breast tissues and 6 breast-cancer cell lines for the presence of splice variants of AR mRNA and have identified an exon 3 deletion splice variant (delta3AR). The higher expression of the variant relative to the wild-type AR (WT AR) was found in 7 breast-cancer samples (delta3/WT > 15%) and relatively lower levels of the variant were observed in 3 breast-cancer cell lines (delta3/WT < 5%). However, in normal breast tissues, expression of the variant was undetectable by Southern blot analysis. In vitro translation of the delta3AR mRNA resulted in a variant AR protein of about 105 kDa, smaller than the WT AR by about 5 kDa. We thus report an exon deletion splice variant of AR mRNA in breast cancer. The variant protein is predicted to lack the second zinc finger within the DNA-binding domain and is expected to be unable or to have reduced ability to bind to androgen-response elements and to activate transcription. The relatively high expression of this AR variant in some breast-cancer tissues may indicate its role in regulating the growth of these cancers.

PMID: 9259393 [PubMed - indexed for MEDLINE]

1: Genes Chromosomes Cancer. 1997 Aug;19(4):256-66.

Related Articles, Links

Differential splicing of exon 5 of the Wilms tumour (WTI) gene.

Renshaw J, King-Underwood L, Pritchard-Jones K.

Section of Paediatrics, Institute of Cancer Research, Surrey, United Kingdom.

The WTI gene encodes a developmentally regulated transcription factor whose function is altered by alternative splicing at two sites: the 17 amino acids of exon 5, whose functional effects are ill-defined, and the 3 amino acids (KTS) between exons 9 and 10, which determine sequence-specific DNA binding and nuclear localisation. Germline mutations, which prevent normal KTS splicing, can underlie the Denys-Drash syndrome, and disruptions of splicing of exon 5 may occur in Wilms tumours. We analysed by reverse transcriptase polymerase chain reaction (RT-PCR) amplification the relative ratios of the four splice variants of WTI mRNA in normal and tumour tissues and found tissue-specific, developmental stage-specific, and species-specific differences in the splicing of exon 5 but not of KTS. We found no evidence for disrupted splicing in acute leukaemias or gonadal tumours. The significance of these findings is discussed, and the possibility is raised that WTI may orchestrate the appropriate response to growth and differentiation factor signalling, mediated by alterations in the relative levels of exon 5 containing WTI isoforms.

PMID: 9258661 [PubMed - indexed for MEDLINE]

1: Anticancer Res. 1997 May-Jun;17(3C):1871-6. Related Articles, Links

Expression of CD44 standard and variant isoforms v5, v6 and v7 in human ovarian cancer cell lines.

Stickeler E, Runnebaum IB, Mobus VJ, Kieback DG, Kreienberg R.

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The expression of standard CD44 protein (CD44std) and its splice variants v5, v6 and v7 was investigated in 43 human ovarian carcinoma cell lines by flow cytometry and immunocytochemistry using monoclonal antibodies raised against extracellular epitopes. Twenty six (60%) cell lines expressed CD44 std. Variant isoforms of CD44 were expressed in 12 of the 26 CD44 positive cell lines. All 12 cell lines expressed CD44 v5. In addition 6 cell lines expressed CD44 v6 and one of these expressed CD44 v7 simultaneously. No significant differences of CD44 expression were found between cell lines derived from solid tumors or ascites. In ovarian cancer cells splicing of CD44 v5 appears to be a prerequisite for expression of downstream variable exons. New acquisition of variable CD44 exons may be implicated in the tumorigenesis of ovarian cancer. The CD44 gene provides a biological model to study the role of alternative splicing in gynecologic malignancy.

PMID: 9216637 [PubMed - indexed for MEDLINE]

1: Hum Genet. 1997 May;99(5):624-7. Related Articles, Links

Mutations of the CD40 ligand gene in 13 Japanese patients with X-linked hyper-IgM syndrome.

Nonoyama S, Shimadzu M, Toru H, Seyama K, Nunoi H, Neubauer M, Yata J, Och HD.

Department of Pediatrics, University of Washington, Seatle 98195, USA.

X-linked hyper-IgM syndrome (XHIM) is a rare primary immunodeficiency caused by a defective CD40 ligand. We identified mutations of the CD40 ligand gene in 13 unrelated Japanese XHIM patients. Of the four patients with missense mutations, one had a mutation within the transmembrane domain, and the three others had mutations affecting the TNF homology region of the extracellular domain. Two of the missense mutations resulted in the substitution of amino acids that are highly conserved in TNF family proteins. Three patients had nonsense mutations, all of which resulted in the truncation of the TNF homology domain of the CD40 ligand. Three patients had genomic DNA deletions of 2, 3 or 4 nucleotides, respectively. All of the deletions were flanked by direct repeat sequences, suggesting that these deletions were caused by slipped mispairing. Three patients had mutations within introns resulting in altered splicing, and multiple splicing products were found in one patient. Thus, each of the 13 Japanese patients had different mutations, 9 of them being novel mutations. These results indicate that mutations in XHIM are highly heterogeneous, although codon 140 seems to be a hot spot of the CD40 ligand gene since two additional point mutations were located at Trp 140, bringing the total numbers of mutations affecting codon 140 to six. In one XHIM family with a missense mutation, prenatal diagnosis was performed by single-strand conformation polymorphism analysis of genomic DNA of a male fetus.

PMID: 9150729 [PubMed - indexed for MEDLINE]

1: Hum Genet. 1997 May;99(5):612-5. Related Articles, Links

Identification of novel mutations in the human EXT1 tumor suppressor gene.

Wells DE, Hill A, Lin X, Ahn J, Brown N, Wagner MJ.

Department of Biology, University of Houston, TX 77204, USA.

Hereditary multiple exostoses (EXT) is a genetically heterogeneous bone disorder caused by genes segregating on human chromosomes 8, 11, and 19 and designated EXT1, EXT2 and EXT3, respectively. Recently, the EXT1 gene has been isolated and partially characterized and appears to encode a tumor suppressor gene. We have identified six mutations in the human EXT1 gene from six unrelated multiple exostoses families segregating for the EXT gene on chromosome 8. One of the mutations we detected is the same 1-bp deletion in exon 6 that was previously reported in two independent EXT families. The other five mutations, in exons 1, 6, 9, and the splice junction at the 3' end of exon 2, are novel. In each case, the mutation is likely to result in a truncated or nonfunctional EXT1 protein. These results corroborate and extend the previous report of mutations in this gene in two EXT families, and provide additional support for the EXT1 gene as the cause of hereditary multiple exostoses in families showing linkage to chromosome 8.

PMID: 9150727 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab. 1997 May;82(5):1345-52.

Related Articles, Links

Tissue-specific expression of alpha and beta messenger ribonucleic acid isoforms of the human mineralocorticoid receptor in normal and pathological states.

Zennaro MC, Farman N, Bonvalet JP, Lombes M.

INSERM U246, Institut Federatif de Recherche Cellules Epitheliales, Faculte de Medecine Xavier Bichat, Paris, France.

Expression of the mineralocorticoid receptor (MR) is restricted to some sodium-transporting epithelia and a few nonepithelial target tissues. Determination of the genomic structure of the human MR (hMR) revealed two different untranslated exons (1alpha and 1beta), which splice alternatively into the common exon 2, giving rise to two hMR mRNA isoforms (hMR alpha and hMR beta). We have investigated expression of hMR transcripts in renal, cardiac, skin, and colonic tissue samples by in situ hybridization with exon 1alpha and 1beta specific riboprobes, using an exon 2 probe as internal control. Specific signals for either exon 1alpha- and 1beta-containing mRNAs were detected in typically hMR-expressing cells in all tissues analyzed. hMR alpha and hMR beta were present in distal tubules of the kidney, in cardiomyocytes, in enterocytes of the colonic mucosa, and in keratinocytes and sweat glands. Interestingly, although both isoforms appear to be expressed at approximately the same level, the relative abundance of each message compared with that of exon 2-containing mRNA strikingly differs among aldosterone target tissues, suggesting the possibility of other tissue-specific transcripts originating from alternative splicing. Finally, functional hypermineralocorticism was associated with reduced expression of hMR beta in sweat glands of two patients affected by Conn's and Liddle's syndrome, whereas normal levels of hMR isoforms were found in one case of pseudohypoaldosteronism. Altogether, our results indicate a differential, tissue-specific expression of hMR mRNA isoforms, hMR beta being down-regulated in situations of positive sodium balance, independently of aldosterone levels.

PMID: 9141514 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab. 1997 Apr;82(4):1160-6. Related Articles, Links

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Altered expression of fibroblast growth factor receptors in human pituitary adenomas.

Abbass SA, Asa SL, Ezzat S.

Department of Pathology, Mount Sinai Hospital, Toronto, Ontario, Canada.

We have shown that basic fibroblast growth factor (FGF) is heterogeneously expressed by human pituitary adenomas and may be implicated as a growth stimulus for these tumors. There are four mammalian FGF receptor (FGFR) genes encoding a complex family of transmembrane tyrosine kinases. The prototypic receptor is composed of three Ig-like extracellular ligand-binding domains, a transmembrane domain, and a cytoplasmic split tyrosine kinase. Multiple forms of cell-bound or secretable isoforms of FGFR-1, -2, and -3 can be generated by cell- and tissue-specific alternative splicing, resulting in tissue-specific FGF function. Shifts in isoform expression accompany tumor progression in some systems. We examined the normal human adenohypophysis and 40 pituitary adenomas to determine the pattern of FGFR expression by reverse transcription-PCR; all tumors were characterized clinically and morphologically. Ribonucleic acid (RNA) was extracted from frozen tumor tissue and primers were used to distinguish messenger RNA of the secretable first Ig-like domain (I) and those of the transmembrane and kinase domains (K) of each FGFR subtype. The normal pituitary-expressed mRNAs for FGFR-1 I and K, FGFR-2 I and K, FGFR-3 I and K, and FGFR-4 I but not FGFR-4 K; this represents the first report of a truncated isoform of FGFR-4, indicating possible alternative polyadenylation sites in this receptor. Only 3 tumors had the same pattern of expression of the 4 FGFRs as the normal gland. Although all tumors expressed FGFR-1 I, 1 tumor did not express FGFR-1 K, suggesting the production of only a secretable form of FGFR-1 by this tumor. Four tumors were negative for FGFR-2 I and K; 6 expressed the secretable form only, and 17 expressed FGFR-2 K but not I. All tumors expressed FGFR-3 I; 14 had secretable forms only, and no tumors expressed FGFR-3 K alone. As in the normal gland, 13 tumors expressed only the secretable I form of FGFR-4. Unlike the normal pituitary, however, 22 expressed FGFR-4 I and K, indicating a possible tumor-specific transmembrane receptor. Five tumors were negative for FGFR-4 I and K. Expression of FGFR proteins was confirmed by immunohistochemical localization of the C-terminal portion of FGFR-1, -2, -3, and -4; the results correlated with the RNA data in each case. There was no correlation between tumor type, size, or aggressiveness and the expression pattern of FGFRs. Our study suggests that pituitary adenomas have altered FGFR subtype and isoform expression, which may determine their hormonal and proliferative responses to FGFs.

PMID: 9100589 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab. 1997 Apr;82(4):1058-65.

Tumor-specific expression of alternatively spliced estrogen receptor messenger ribonucleic acid variants in human pituitary adenomas.

Chaidarun SS, Klibanski A, Alexander JM.

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The well documented mitogenic and hormone regulatory effects of estrogen (E2) on pituitary cells are mediated via its nuclear receptor (ER), a cellular homolog of v-erbA oncogene. ER isoforms generated by alternative exon splicing, termed ER variants delta 2ER to delta 7ER, have been identified in breast cancer and have been postulated to have important pathogenetic and clinical implications in tumorigenesis and/or development of hormone resistance. Because pituitary tumors, particularly prolactinomas, are known to be E2-dependent, we investigated alternatively spliced ER variant messenger ribonucleic acid expression in 40 human pituitary tumors of various phenotypes and normal pituitary tissues, using reverse transcription-PCR and Southern blot analyses. Nine of 11 prolactinomas readily expressed multiple ER variants (delta 2ER, delta 4ER, 5ER, and delta 7ER), whereas 6 of 11 tumors showed faint expression of delta 3ER. Four of 7 glycoprotein hormone-producing tumors that synthesized FSH beta expressed delta 2ER, delta 5ER, and delta 7ER. In 9 GH- and 10 ACTH-secreting tumors examined, the expression of normal and variant ER was restricted to tumors that also exhibited scattered PRL immunoreactivity. Variant and normal ER were not found in three null cell tumors (oncocytomas) that showed negative immunoreactivity for all pituitary hormones or their subunits. In contrast, only delta 4ER and delta 7ER were uniformly detected in normal pituitaries. delta 6ER was not detected in any normal or neoplastic pituitary specimen studied. We conclude that multiple alternatively spliced ER variants are coexpressed with normal ER in a tumor phenotype-specific manner. In addition, ER variants delta 2ER and delta 5ER were found to be tumor specific. Future functional studies will be required to determine whether coexpression of multiple ER variants along with normal ER confers a pathophysiological role in pituitary hormone regulation and/or tumor cell proliferation.

PMID: 9100573 [PubMed - indexed for MEDLINE]

1: Lung Cancer. 1997 Mar;16(2-3):151-72. Related Articles, Links

Preferential histiotypic expression of CD44-isoforms in human lung cancer.

Wimmel A, Schilli M, Kaiser U, Havemann K, Ramaswamy A, Branscheid D, Kogan E, Schuermann M.

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The CD44 transmembrane glycoprotein is expressed in most adult tissues and in the majority of neoplasias. Due to alternative splicing, this cell adhesion molecule exists in multiple isoforms some of which have been associated with specific types of tumours as well as with increased tumour metastasis. In this study, we have looked at the level and type of CD44 expression in lung cancer which represents a histologically heterogenous form of cancer composed of small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC), the latter subgroup comprising adenocarcinoma (ADC), bronchio-alveolar carcinoma (BAC), large cell carcinoma (LCC), and squamous cell carcinoma (SCC). We analysed 20 lung cancer cell lines and 64 primary tumours by RT-PCR and immunohistochemical detection of the CD44 standard and variant protein isoforms. Our results suggest that (i) CD44 is expressed in all histologically distinct subsets of lung cancer with a tendency SCC > BAC > ADC > LCC > SCLC, (ii) expression of the CD44 isoforms v5, v7, v8, and, most notably that of CD44 exon v6, strongly correlates with tumours of squamous cell and bronchio-alveolar carcinoma origin, tumours which commonly exhibit a comparatively low metastasizing potential, and (iii) the expression of CD44 isoforms is independent from the tumour size and lymph node status at surgery, the proliferative status of the tumour cell population (Ki67 antigen expression) and the histopathological grading (G1 to G3). Only non-differentiated tumours (G4), which were restricted to SCLC and LCC samples revealed markedly reduced CD44 standard and isoform antigen. In conclusion, our data point to a clear histiotype-related pattern of CD44 variant expression preferentially that of CD44v6 in SCC and BAC.

PMID: 9152947 [PubMed - indexed for MEDLINE]

1: Anal Biochem. 1997 Feb 15;245(2):167-78.

Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Quantitation of estrogen receptor mRNA and its alternatively spliced mRNAs in breast tumor cells and tissues.

Fasco MJ.

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Estrogen receptor (ER) mRNA exists as wild-type (full-length) and alternatively spliced variants in cell lines, normal tissues, and tumors. Most of the alternatively spliced variants discovered so far are missing one or more complete exons. RNase protection and RNA-PCR assays used previously to determine the relative concentration of a particular ER spliced-variant mRNA to wild-type mRNA have produced equivocal results because the probes/primers targeted only small regions within the nucleotide sequence. Variant ER mRNAs missing an exon outside the probe/primer region will react as if they were wild-type and any alternatively spliced variants containing a deletion at the probe/primer annealing site(s) will not be detected. A highly sensitive, competitive RNA-PCR assay has been developed that is quantitative with respect to the relative composition of wild-type ER and its alternatively spliced-mRNA forms, and semiquantitative with respect to their concentrations in cells and tissues. Separation and quantitation of the products are rapidly and accurately achieved by, respectively, capillary electrophoresis and laser-induced fluorescence. Wild-type ER mRNA concentration can be measured independently of all the reported exon deletion forms in a single PCR assay. Specific exon deletion forms can be measured by ER cDNA amplification with overlapping primer sets. Results obtained with RNAs isolated from two MCF-7 cell lines, a T-47D cell line, and five breast tumor tissues are presented.

PMID: 9056208 [PubMed - indexed for MEDLINE]

1: Oncogene. 1997 Feb 6;14(5):617-21. Related Articles, Links

nature publishing group

Frequent association of alternative splicing of NER, a nuclear hormone receptor gene in cancer tissues.

Saito H, Nakatsuru S, Inazawa J, Nishihira T, Park JG, Nakamura Y.

Department of Human Genome Analysis, Cancer Institute, Toshima-ku, Tokyo, Japan.

We have detected frequent alternative splicing of a gene that encodes NER, a protein homologous to the retinoic acid receptors, in cancer cells. Western and immunohistochemical analyses disclosed accumulation of a large amount of the aberrant NER product, generated by alternative splicing that caused skipping of an exon corresponding to the DNA-binding domain, in the nucleoli of cells of cancer cell lines and primary cancer tissues. The aberrant protein was detected in 116 of 228 primary cancers developed in various tissues including breast and colon, but was absent in the corresponding normal tissues; it was also detected in 31 of 39 cancer cell lines. This observation may imply that the aberrant NER product has some relation to the development and/or progression of cancers in a variety of human tissues.

PMID: 9053861 [PubMed - indexed for MEDLINE]

1: Br J Cancer. 1997;75(2):268-74. Related Articles, Links

Spontaneous overexpression of the long form of the Bcl-X protein in a highly resistant P388 leukaemia.

Kuhl JS, Krajewski S, Duran GE, Reed JC, Sikic BI.

Department of Medicine, Stanford University School of Medicine, CA 94305-5306, USA.

A novel resistant variant of murine P388 leukaemia, P388/SPR, was identified by de novo resistance to doxorubicin (DOX) in vivo. This mutant displayed a similar level of cross-resistance to etoposide (VP-16) and other topoisomerase II (topo II) inhibitors. Further analysis of the phenotype revealed a broad cross-resistance to vinca alkaloids, alkylating agents, antimetabolites, aphidicolin and UV light. Low-level expression of mdr1 and P-glycoprotein (P-gp), as well as a modest impairment of cellular drug accumulation and partial reversion of resistance to DOX and VP-16 by cyclosporine, confirmed a moderate role of P-gp in conferring drug resistance in P388/SPR cells. Consistent changes in neither topo II expression or activity nor glutathione metabolism could be detected. Induction of apoptosis was significantly reduced in P388/SPR cells, as indicated by minimal DNA fragmentation. Analysis of oncogenes regulating apoptotic cell death revealed a marked decrease of bcl-2 in combination with a moderate reduction of bax protein, but a striking overexpression of the long form of the bcl-X protein. Transfection of human bcl-X-L into P388 cells conferred drug resistance similar to that of P388/SPR cells. The data suggest that overexpression of bcl-X-L results in an unusual phenotype with broad cross-resistance to non-MDR-related cytotoxins in vitro, and provide an interesting example of spontaneous overexpression of another member of the bcl-2 gene family in cancer.

PMID: 9010037 [PubMed - indexed for MEDLINE]

1: Neuroscience. 1997 Jan;76(1):187-202. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Alterations in hippocampal expression of SNAP-25, GAP-43, stannin and glial fibrillary acidic protein following mechanical and trimethyltin-induced injury in the rat.

Patanow CM, Day JR, Billingsley ML.

Department of Pharmacology, Pennsylvania State University College of Medicine, Milton S. Hershey Medical Center, Hershey 17033, USA.

A set of well-defined antisera against neuronal and glial proteins were used to characterize patterns of protein expression in rat hippocampus following transection of the fimbira-fornix and perforant pathways or after administration of the selective neurotoxicant trimethyltin (8 mg/kg, i.p.). SNAP-25 (synaptosomal protein, mol. wt 25,000) is a neuron-specific, developmentally regulated presynaptic protein, stannin is a protein enriched in cells sensitive to trimethyltin, and GAP-43 (growth-associated protein, mol. wt 43,000) is associated with axonal growth and regeneration. Glial fibrillary acidic protein is an astrocyte-specific intermediate filament protein and a marker for reactive gliosis. SNAP-25 immunoreactivity was altered following both neurotoxicant and mechanical injury. Three days after fimbria-fornix/perforant path lesions, there was a loss of SNAP-25 immunoreactivity in hippocampal efferent pathways and in the lesioned entorhinal cortex. By day 12, there was evidence of reinnervation of hippocampal subfields by SNAP-25-immunopositive commissural afferent fibers. On day 3, immunoblots showed the appearance of SNAP-25a, a developmental isoform produced by alternative splicing of nine amino acids in exon 5, in lesioned tissues. This isoform declined by day 12 and was not found in contralateral control hippocampus or non-lesioned brain regions. Stannin immunoreactivity was unchanged, while GAP-43 was prominent on day 12 post-lesion. Glial fibrillary acidic protein immunoreactivity indicated gliosis near the site of pathway transection. In contrast, trimethyltin induced a marked loss of stannin immunoreactivity in hippocampal neurons seven days after injection. Trimethyltin increased glial fibrillary acidic protein staining in the hippocampus and other damaged regions. SNAP-25 immunoreactivity was markedly increased in mossy fibers and other hippocampal fields seven days following trimethyltin. Immunoblot analysis showed that only the adult SNAP-25b isoform was expressed after trimethyltin intoxication. These data suggest that SNAP-25 is a useful marker for presynaptic damage. Furthermore, reexpression of developmental isoforms of SNAP-25a may precede functional reinnervation when the postsynaptic target remains intact.

PMID: 8971771 [PubMed - indexed for MEDLINE]

1: Cancer Res. 1996 Nov 15;56(22):5128-31. Related Articles, Links

Frequent abnormalities of FHIT, a candidate tumor suppressor gene, in head and neck cancer cell lines.

Mao L, Fan YH, Lotan R, Hong WK.

Department of Thoracic/Head & Neck Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston 77030, USA.

Loss of heterozygosity at the short arm of chromosome 3 occurs frequently in head and neck squamous cell carcinoma (HNSCC). FHIT, a candidate tumor suppressor gene, was recently identified at 3p14.2, and abnormalities of the gene were found in several types of human cancers. To investigate a potential role of the FHIT gene in HNSCC, we examined 16 HNSCC cell lines from 11 patients for abnormalities of the gene by using microsatellite analysis, reverse transcription-PCR, sequencing, and Southern blot analysis. We found that 13 of 16 (81%) cell lines exhibit loss of heterozygosity at 3p14.2. Seven cell lines from six individuals exhibited abnormal transcription patterns, including lack of a FHIT transcript in three lines and shortened transcripts in four lines. A further examination of coding sequences of FHIT in all lines with FHIT transcripts revealed a deletion of exon 4 in one line, a deletion of exons 5 to 7 in one line, and a deletion of exons 5 to 7 plus multiple small insertions between exons 4 and 8 in two lines derived from a primary tumor and a metastasis in the same individual. These results indicate that FHIT may have been inactivated in six cell lines from five (45%) individuals. We also observed two common polymorphism sites at codons 88 and 98 of the gene. These data indicate that abnormal transcription of the FHIT gene is common in HNSCC cell lines; however, other tumor suppressor gene(s) may reside at the same chromosomal region.

PMID: 8912845 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1996 Nov 8;271(45):28617-23.

Alternative splicing in COL1A1 mRNA leads to a partial null allele and two In-frame forms with structural defects in non-lethal osteogenesis imperfecta.

Wang Q, Forlino A, Marini JC.

Section on Connective Tissue Disorders, Heritable Disorders Branch, NICHD, National Institutes of Health, Bethesda, Maryland 20892-1830, USA.

We have identified a novel multiexon genomic deletion in one COL1A1 collagen allele that results in three alternative forms of mutant mRNA. This mutation occurs in a 9-year-old girl and her father, both affected with severe type III osteogenesis imperfecta (OI). We previously reported detection of a mismatch in their alpha1(I) amino acids 558-861 region by RNA/RNA hybrid analysis (Grange, D. K., Gottesman, G. S., Lewis, M. B., and Marini, J. C. (1990) Nucleic Acids Res. 18, 4227-4236). Single Strand Conformational Polymorphism further localized the mRNA mutation to the amino acids 579-679 coding region. At the gene level, polymerase chain reaction (PCR) amplification of patient leukocyte DNA from the exon 33-38 region yielded the normal 1004-base pair (bp) fragment and an additional 442-bp fragment. Sequencing of the shorter genomic PCR product confirmed the presence of a 562-bp deletion, extending from the last 3 nucleotides (nt) of exon 34 to 156 nt from the 3'-end of intron 36. The genomic deletion was also detected in the clinically normal grandmother, who was confirmed to be a mosaic carrier. PCR amplification and RNase protection experiments were used to investigate the mRNA structure and occurrence of alternative splicing. One form of the mutant cDNA has a deletion with end points that are identical to the genomic deletion. This results in a combination deletion/insertion, with a deletion of amino acids 603-639 followed by an insertion of 156 nt from the 3'-end of intron 36. In addition, we found two alternatively spliced forms. One form uses a cryptic donor site in exon 34 and the exon 37 acceptor. The second form uses the normal exon 32 splice donor and exon 37 acceptor. Use of the cryptic donor results in a coding sequence that is out-of-frame. Both the retained intron form and the use of the exon 32 donor site result in coding sequences that are in-frame. This is the first report of a collagen defect in OI with alternative splicing generating both in-frame and out-of-frame forms of mRNA. Although the in-frame forms constitute more than 60% of the mRNA from the mutant allele, no mutant protein chain was identified. Collagen produced by cultured OI osteoblasts showed a significant increase in the relative amount of type III collagen but no mutant alpha1(I) chain.

Publication Types:

- Case Reports

PMID: 8910493 [PubMed - indexed for MEDLINE]

1: Br J Cancer. 1996 Oct;74(7):999-1004. Related Articles, Links

Expression of the extracellular matrix protein tenascin in malignant and benign ovarian tumours.

Wilson KE, Langdon SP, Lessells AM, Miller WR.

ICRF Medical Oncology Unit, Western General Hospital, Edinburgh, UK.

The extracellular matrix protein tenascin (TN) is overexpressed in a number of solid tumours. Thi however, is the first study to examine TN expression in ovarian tumours. TN protein was examined in froze sections of 50 human ovarian tumours by immunohistochemistry. Malignant and borderline tumours showed significantly greater incidence and intensity of stromal staining than benign tumours ($P < 0.0001$ and $P = 0.038$ respectively). Seven omental metastases were also examined and showed a strikingly similar protein distribution to their primary tumour counterparts. The expression pattern of different RNA isoforms, created by alternative splicing of the primary transcript, was identified using reverse transcription-polymerase chain reactions (RT-PCR). The smallest TN RNA splice variant (284 bp) was found in all tumours examined, while the appearance of larger molecular weight transcripts (approximately 490 and 556 bp), as major forms, was predominantly limited to malignant tumours, with 9/12 malignant tumours showing this pattern compared with 1/6 benign tumours. These data suggest that malignant ovarian tumours have increased expression of TN compared with benign tumours and this may be associated with induction of specific isoforms.

PMID: 8855965 [PubMed - indexed for MEDLINE]

1: Jpn J Cancer Res. 1997 Apr;88(4):385-8. Related Articles, Links

Alternative splicing of the FHIT gene in colorectal cancers.

Hibi K, Taguchi M, Nakamura H, Hirai A, Fujikake Y, Matsui T, Kasai Y, Akiyama S, Ito K, Takagi H.

Second Department of Surgery, Nagoya University School of Medicine, Showa-ku.

In the present study, we examined the status of the FHIT gene in 112 colorectal cancer and 137 colorectal adenoma specimens. In a total of 5 specimens (4 colorectal cancers and 1 colorectal adenoma), a common smaller product was detected in addition to the normal size product. This smaller product had lost exon 4, the 5' noncoding region of the FHIT gene, owing to alternative splicing. Moreover, all of the 5 tumors with alternative splicing were located lower on the rectum than the anterior peritoneal reflection.

PMID: 9197530 [PubMed - indexed for MEDLINE]

1: Am J Hum Genet. 1996 Mar;58(3):491-8. Related Articles, Links

FGFR2 exon IIIa and IIIc mutations in Crouzon, Jackson-Weiss, and Pfeiffer syndromes: evidence for missense changes, insertions, and a deletion due to alternative RNA splicing.

Meyers GA, Day D, Goldberg R, Daentl DL, Przylepa KA, Abrams LJ, Graham JM Jr, Feingold M, Moeschler JB, Rawnsley E, Scott AF, Jabs EW.

Center for Medical Genetics, Johns Hopkins Hospital, Baltimore, MD 21287-3914, USA.

Fibroblast growth factor receptor 2 (FGFR2) mutations have been associated with the craniosynostotic conditions Crouzon, Jackson-Weiss, and Pfeiffer syndromes. Previously, mutations were described in the exons IIIa and IIIc, which form the extracellular, third immunoglobulin-like domain (IgIII) and adjacent linker regions, both of which are normally involved in ligand binding. For all three conditions, mutations were found in exon IIIc. Only in Crouzon syndrome were mutations identified in exon IIIa. In this study, 39 cases with one of these three conditions were screened for exon IIIa or IIIc mutations. Eleven mutations are reported in 17 unrelated cases. Mutations in exon IIIa are identified for not only Crouzon but also Jackson-Weiss and Pfeiffer syndromes. Four mutations in either exon IIIa or exon IIIc reported only in Crouzon syndrome are present also in one of the other two syndromes. Two insertions, one in exon IIIa in a Crouzon syndrome patient and the other in exon IIIc in a Pfeiffer syndrome patient, were observed. The latter mutation has the same alternative RNA splicing effect as a reported synonymous mutation for Crouzon syndrome. A missense mutation was detected in one Pfeiffer syndrome family in which two members had craniosynostosis without limb anomalies. The inter- and intrafamilial variability in expression of FGFR2 mutations suggests that these three syndromes, presumed to be clinically distinct, are instead representative of a spectrum of related craniosynostotic and digital disorders.

PMID: 8644708 [PubMed - indexed for MEDLINE]

1: J Exp Med. 1996 Mar 1;183(3):1131-40. Related Articles, Links

Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen.

Wang RF, Parkhurst MR, Kawakami Y, Robbins PF, Rosenberg SA.

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Tumor infiltrating lymphocytes (TILs) derived from tumor-bearing patients recognize tumor-associated antigens presented by major histocompatibility complex (MHC) class I molecules. The infusion of TIL586 along with interleukin (IL) 2 into an autologous patient with metastatic melanoma resulted in the objective regression of tumor. A gene encoding a tumor antigen recognized by TIL586 was recently isolated and shown to encode gp75. Here we report that an antigenic peptide, MSLQRQFLR, recognized by TIL586 was not derived from the normal gp75 protein. Instead, this nonamer peptide resulted from translation of an alternative open reading frame of the same gene. Thus, the gp75 gene encodes two completely different polypeptides, gp75 as an antigen recognized by immunoglobulin G antibodies in sera from a patient with cancer, and a 24-amino acid product as a tumor rejection antigen recognized by T cells. This represents the first demonstration that a human tumor rejection antigen can be generated from a normal cellular gene using an open reading frame other than that used to encode the normal protein. These findings revealed a novel mechanism for generating tumor antigens, which may be useful as vaccines to induce tumor-specific cell-mediated immunity against cancer.

PMID: 8642255 [PubMed - indexed for MEDLINE]

1: DNA Cell Biol. 1996 Mar;15(3):175-85. Related Articles, Links

A large variety of alternatively spliced and differentially expressed mRNAs are encoded by the human acute myeloid leukemia gene AML1.

Levanon D, Bernstein Y, Negreanu V, Ghozi MC, Bar-Am I, Aloya R, Goldenberg D, Lotem J, Groner Y.

Department of Molecular Genetics and Virology, The Weizmann Institite of Science, Rehovot, Israel.

The human chromosome 21 acute myeloid leukemia gene AML1 is frequently rearranged in the leukemia-associated translocations t(8;21) and t(3;21), generating fused proteins containing the amino-terminal part of AML1. In normal blood cells, five size classes (2-8 kb) of AML1 mRNAs have been previously observed. We isolated seven cDNAs corresponding to various AML1 mRNAs. Sequencing revealed that their size differences were mainly due to alternatively spliced 5' and 3' untranslated regions, some of which were vast, exceeding 1.5 kb (5') and 4.3 kb (3'). These untranslated regions contain sequences known to control mRNA translation and stability and seem to modulate AML1 mRNA stability. Further heterogeneity was found in the coding region due to the presence of alternatively spliced stop codon-containing exons. The latter led to production of polypeptides that were smaller than the full-length AML1 protein; they lacked the trans-activation domains but maintained DNA binding and heterodimerization ability. The size of these truncated products was similar to the AML1 segment in the fused t(8;21) and t(3;21) proteins. In thymus, only one mRNA species of 6 kb was detected. Using in situ hybridization, we showed that its expression was confined to the cortical region of the organ. The 6-kb mRNA was also prominent in cultured peripheral blood T cells, and its expression was markedly reduced upon mitogenic activation by phorbol myristate acetate (TPA) plus concanavalin A (ConA). These results and the presence of multiple coding regions flanked by long complex untranslated regions, suggest that AML1 expression is regulated at different levels by several control mechanisms generating the large variety of mRNAs and protein products.

PMID: 8634147 [PubMed - indexed for MEDLINE]

1: Br J Haematol. 1996 Mar;92(4):900-6. Related Articles, Links

Predominant expression of the long isoform of Bcl-x (Bcl-xL) in human lymphomas.

Xerri L, Parc P, Brousset P, Schlaifer D, Hassoun J, Reed JC, Krajewski S, Birnbaum D.

Departement de Pathologie, Institut Paoli-Calmettes, Marseille, France.

Bcl-x is a member of the bcl-2 family of proteins which are characterized by their ability to modulate apoptosis. Alternative splicing results in two distinct bcl-x mRNAs encoding a long isoform, bcl-xL, which acts as a bcl-2 agonist; and a short isoform, bcl-xS, which inhibits bcl-2 effects. The aim of the study was to determine whether bcl-x is expressed in lymphoma tissues and to characterize the respective production of bcl-xs and bcl-xL. We investigated the expression of bcl-x mRNA in a series of 50 non-Hodgkin's lymphomas (NHL) and Hodgkin's disease (HD) cases using a RT-PCR method in order to amplify both transcripts simultaneously, and to estimate their relative abundance. The rearrangements of the bcl-2 gene were analysed by RT-PCR expression of the hybrid bcl-2-lgH mRNA. In addition, 20 PCR-positive NHL cases and three HD cases were analysed by immunohistochemistry using bcl-x polyclonal antisera. RT-PCR showed bcl-x expression in 43/45 NHLs and 5/5 HD cases. The bcl-xL transcript was predominant in all positive cases and was associated with variable amounts of bcl-xS. There was no significant correlation between the profile of bcl-xL/bcl-xS expression and the histological and immunological subtyping. Bcl-x immunodetection was positive in the neoplastic cell component in all analysed cases, but the degree of staining was highly variable between cases. Expression of the hybrid bcl-2-IgH gene was detected by RT-PCR in five cases of follicular NHL and in one case of HD, but this group of tumours did not display a particular profile of bcl-xL/bcl-xS expression. We conclude that bcl-x is commonly expressed by malignant cells in various types of malignant lymphomas, with a predominance of the bcl-xL transcript. Since the corresponding bcl-xL isoform can block the cell death machinery and potentialize bcl-2 effects, it may be involved in some pathways of lymphomagenesis.

PMID: 8616083 [PubMed - indexed for MEDLINE]

1: Arch Biochem Biophys. 1996 Mar 1;327(1):35-40.

Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Retarded and aberrant splicings caused by single exon mutation in a phosphoglycerate kinase variant.

Ookawara T, Dave V, Willems P, Martin JJ, de Barsy T, Matthys E, Yoshida A.

Department of Biochemical Genetics, Beckman Research Institute of the City of Hope, Duarte, California 91010, USA.

The molecular abnormality of a phosphoglycerate kinase variant which was associated with severe tissue enzyme deficiency and episodes of muscle contractions and myoglobinuria was examined. Analysis of the patient's DNA showed the existence of a nucleotide transversion A/T - C/G in exon 7. No other nucleotide change was detected in the coding region of the variant gene. The mutation should produce a single amino acid substitution Glu - Ala at protein position 251 counting from the NH2-terminal acetyl serine residue. The protein abnormality caused by the amino acid substitution cannot explain the enzyme deficiency. Northern blot hybridization indicated that the PGK mRNA content of the patient's lymphoblastoid cells was only about 10% of that of normal. Nucleotide sequence analysis revealed the existence of two PGK mRNA components in the patient's cells. The major component corresponds to the normal PGK mRNA except for A - C change at nucleotide position 755 counting from adenine of the chain initiation codon. The minor component contains 5' region (52 bases) of intron 7 between exon 7 and exon 8. An inframe chain termination codon exists in the minor mRNA component, and the COOH-terminal half is expected to be deleted in the translation product. These results indicate that the low PGK activity in the patient's tissues is mainly due to retarded and aberrant pre-mRNA splicings caused by the change of the consensus 5' splice sequence AGgt to a nonconsensus sequence CGgt at the junction between exon 7 and intron 7 of the variant gene.

Publication Types:

- Case Reports

PMID: 8615693 [PubMed - indexed for MEDLINE]

1: Neurosurgery. 1996 Feb;38(2):362-6.

Related Articles, Links

Expression and alternative splicing of Pit-1 messenger ribonucleic acid in pituitary adenomas.

Hamada K, Nishi T, Kuratsu J, Ushio Y.

Department of Neurosurgery, Kumamoto University Medical School, Kumamoto City, Japan.

Twenty-eight human pituitary adenomas were analyzed for the expression of Pit-1 messenger ribonucleic acid (mRNA) by using reverse transcriptase-polymerase chain reaction analysis of frozen-section mRNA. Pit-1 mRNA was detected in all functioning tumors and in 9 of 11 nonfunctioning tumors. Pit-1 beta, which is a more active isoform of transcriptional factor for growth hormone than Pit- alpha and which arises from an alternative splicing mechanism, was detected in 14 of 17 functioning tumors and in 5 of 11 nonfunctioning tumors. The transcript that corresponds to Pit-1T, which increases thyroid-stimulating hormone beta promoter activity in rat thyrotropic tumor cells, was not found. There was no significant difference in the total Pit-1 (alpha+beta) mRNA expression level between functioning tumors and nonfunctioning tumors. Growth hormone-producing tumors and other pituitary adenomas also showed no significant difference in the Pit-1 beta/Pit-1 alpha expression ratio. Our data suggest that the major role of Pit-1 gene in pituitary adenoma might not be involved in the regulation of hormone production.

PMID: 8869065 [PubMed - indexed for MEDLINE]

1: Leukemia. 1996 Feb;10(2):204-6. Related Articles, Links

Acute myelogenous leukemia: a disorder of gene splicing?

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Department of Human Genetics, Sylvius Laboratories, Leiden University, The Netherlands.

The two common rearrangements t(8;21) and inv(16) are found in approximately 20% of all acute myelogenous leukemias. Both aberrations result in the formation of a fusion gene, involving subunits of the core binding transcription factor (CBF). In this manuscript we hypothesize that the alternative splicing of the fusion genes, leading to truncated CBF subunits, contributes to the pathogenesis of t(8;21) and inv(16) leukemias.

PMID: 8637227 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab. 1996 Feb;81(2):783-90. Related Articles, Links

Tumor-specific expression and alternate splicing of messenger ribonucleic acid encoding activin/transforming growth factor-beta receptors in human pituitary adenomas.

Alexander JM, Bikkal HA, Zervas NT, Laws ER Jr, Klibanski A.

Department of Medicine, Massachusetts General Hospital, Boston 02114, USA.

Activin, a member of the transforming growth factor-beta (TGF beta) cytokine family, acts as a pituitary cell mitogen via a novel family of receptor-linked serine/threonine (Ser/Thr) kinases. Pituitary tumors synthesize activin subunits, and the autocrine action of these growth factors may modulate tumor proliferation. We, therefore, investigated the expression of activin/TGF beta type I receptor messenger ribonucleic acids (mRNAs), designated ALK1 through ALK5 (ALK = activin receptor-like kinase), and type II receptor mRNAs using RT-PCR in 34 human pituitary adenomas of all phenotypes and normal pituitary tissue. ALK2 and ALK5, specific mediators of activin and TGF beta signals, respectively, were found to be expressed only in tumor and not in normal pituitary cells, and ALK2 expression was found only in tumors of a mammosomatotroph cell lineage. ALK1, ALK3, and ALK4 mRNAs were found in both normal and neoplastic pituitary cells. The alternatively spliced cytoplasmic domain of ALK4 consists of 11 kinase subdomains, that are critical for modulating receptor function and intracellular signaling. Truncated forms of the ALK4 cytoplasmic domain lacking these subdomains may attenuate activin signal transduction and affect both tumor phenotype and proliferation via the formation of inactive type I/type II complexes. Three truncated ALK4 receptor mRNAs generated by alternate splicing of the cytoplasmic Ser/Thr kinase domain were found to be tumor specific. One of these truncated receptor mRNAs, ALK4-5, is a novel splice variant that has not been previously described. Expression of the ActRII and T beta RII type II receptor mRNAs, which specifically bind activin and TGF beta, respectively, was highly prevalent among all tumor subtypes and normal pituitary tissue. However, ActRIIB, an activin-specific type II receptor that displays a 3- to 4-fold higher affinity for ligand than ActRII, was expressed in 94% of tumors, but was not prevalent in normal tissue. These data are the first to demonstrate tumor-specific expression of Ser/Thr kinase receptors mRNAs and their splice variants in human pituitary adenomas.

PMID: 8636304 [PubMed - indexed for MEDLINE]

1: Diabetologia. 1996 Feb;39(2):220-5. Related Articles, Links

Chronic primary hyperinsulinaemia is associated with altered insulin receptor mRNA splicing in muscle of patients with insulinoma.

Sbraccia P, D'Adamo M, Leonetti F, Caiola S, Iozzo P, Giaccari A, Buongiorno A, Tamburrano G.

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Alternative splicing of the 36-base pair exon 11 of the human insulin receptor gene results in the synthesis of two insulin receptor isoforms with distinct functional characteristics (the isoform containing exon 11 has lower insulin binding affinity and lower internalization rate). Altered expression of these insulin receptor isoforms has been previously demonstrated in skeletal muscle of patients with non-insulin-dependent diabetes mellitus (NIDDM). However, this observation was not confirmed by other studies and is still a matter of controversy; furthermore, it is not known whether it represents a primary event or is secondary to hyperinsulinaemia and insulin resistance. In order to address this issue in patients with pure non-genetically determined hyperinsulinaemia, we examined the alternative splicing of insulin receptor mRNAs in skeletal muscle of eight patients with surgically confirmed insulinoma and insulin resistance and in eight healthy subjects, using the reverse transcriptase-polymerase chain reaction technique. The insulinoma patients displayed a significant increase in the expression of the insulin receptor isoform containing exon 11 (75.7 +/- 2.3%) when compared with normal subjects (57.9 +/- 1.5%); furthermore, this increase was positively correlated with plasma insulin concentration and negatively correlated with in vivo insulin sensitivity (glucose clamp). In conclusion, the increased expression of the insulin receptor isoform with lower insulin binding affinity in patients with primary non-genetically determined hyperinsulinaemia supports a role for insulin in the regulation of alternative splicing of insulin receptor pre-mRNA and suggests that in NIDDM an altered receptor isoform distribution might be secondary to the ambient hyperinsulinaemia rather than representing a primary defect.

PMID: 8635675 [PubMed - indexed for MEDLINE]

1: Am J Pathol. 1996 Feb;148(2):579-92.

Related Articles, Links

Expression of fibronectin ED-A+ and ED-B+ isoforms by human and experimental colorectal cancer. Contribution of cancer cells and tumor-associated myofibroblasts.

Pujuguet P, Hammann A, Moutet M, Samuel JL, Martin F, Martin M.

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Alternative splicing of primary fibronectin (FN) mRNA results in the synthesis of different isoforms. ED-A+ and ED-B+ FN isoforms are absent from plasma FN and are representative of cellular FN. Their expression was studied in human and rat normal colon, in human colorectal carcinomas, and in transplanted tumors derived from a chemically-induced rat colon cancer. In normal colon, only the ED-A+ FN isoform was expressed as a thin deposit between crypt colonocytes and pericryptal myofibroblasts. Conversely, heavy ED-A+ FN deposits and lighter ED-B+ FN expression were found in the stroma of colorectal tumors in association with myofibroblasts surrounding tumor glands. Some colonic cancer cells also contained intracellular FN isoform granules and expressed FN mRNA. Tumor-associated myofibroblasts and some cancer cell lines were able to synthesize and deposit extracellular ED-A+ and ED-B+ FN in vitro. FN isoform deposition by tumor-associated myofibroblasts was not modulated by colon cancer cell-conditioned medium, but was strongly enhanced when myofibroblasts were cultured on colon cancer cell extracellular matrix or on laminin. These results show that the ED-A+ and ED-B+ FN isoforms were overexpressed in colorectal cancer. Cancer cells can deposit these FN isoforms directly and also stimulate their deposition by tumor-associated myofibroblasts.

PMID: 8579120 [PubMed - indexed for MEDLINE]

1: Hum Genet. 1996 Feb;97(2):198-203. Related Articles, Links

Human Genetics

Mutations in the iduronate-2-sulfatase gene in five Norwegians with Hunter syndrome.

Olsen TC, Eiken HG, Knappskog PM, Kase BF, Mansson JE, Boman H, Apold J.

Department of Medical Genetics, Haukeland Hospital, University of Bergen, Norway.

We have identified the mutations in the iduronate-2-sulfatase (IDS) gene of five unrelated Norwegians with Hunter syndrome by reverse transcription-polymerase chain reaction (RT-PCR) analysis of IDS mRNA followed by single strand conformation polymorphism (SSCP) analysis and cDNA sequencing. One patient had a 5-bp deletion, located at the intron 5/exon 6 junction, that created a new alternative splice site. This expanded the deletion to 9 bp in mRNA, an in-frame deletion of the first 3 codons of exon 6 of the IDS gene. In two patients point mutations were identified, the S333L mutation, which has been reported previously, and A346D (a C-->A transversion at nucleotide 1161/exon 8), which is novel. Two patients had large 3' mRNA rearrangements. The A346D mutation was associated with the mild phenotype, all others with the severe form.

PMID: 8566953 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1996 Jan 26;271(4):2271-8. Related Articles, Links

A novel meprin beta' mRNA in mouse embryonal and human colon carcinoma cells.

Dietrich JM, Jiang W, Bond JS.

Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey 17033, USA.

Meprins, metalloendopeptidases of the astacin family, are composed of alpha and/or beta subunits and are expressed at high levels in mammalian renal and intestinal brushborder membranes. Only one mRNA has been identified previously for each of the subunits in adult human and rodent tissues; a 3.6-kilobase message for the alpha subunit and a 2.5-kilobase message for the beta subunit. The present study reports that a larger beta subunit message (2.7 kilobases, referred to as beta'), and no alpha subunit message, is expressed in embryonal carcinoma cell lines, F9 and Nulli-SSC1, and in human colon adenocarcinoma cells, HT-28-18-C1. Furthermore, in Nulli-SSC1 cells, the beta isoform is induced by the morphogen retinoic acid. The beta' isoform differs from beta only in a portion of the 5'-coding (corresponding to the signal and prosequence domains of the protein) and noncoding region. Only one gene was found for the beta subunit in the mouse and human genome. The deduced amino acid sequence of beta' has no homology with beta in the first 35 NH2-terminal residues, but the two sequences are identical after that. In vitro translation experiments indicated that the size of the protein product of beta' cDNA was similar to that of the beta cDNA protein product, and, in the presence of microsomal membranes, both were glycosylated. These studies indicate that the messages for the meprin beta and beta' subunit result from differential promoter usage and alternate splicing. Expression of the two isoforms may be regulated differentially depending on cell type and/or differentiation state of the cell.

PMID: 8567689 [PubMed - indexed for MEDLINE]

1: DNA Seq. 1996;6(5):263-73. Related Articles, Links

DENN, a novel human gene differentially expressed in normal and neoplastic cells.

Chow VT, Lee SS.

Department of Microbiology, Faculty of Medicine, National University of Singapore, Singapore.

A novel 5843 bp human cDNA sequence was isolated from fetal liver cDNA using the RACE procedure. Within this cDNA was characterised an uninterrupted ORF of 3861 nt encoding a predicted hydrophilic protein of 1287 aa with a calculated molecular mass of 141790 Da and an isoelectric point of 5.41. An RGD cellular adhesion motif was identified within the putative serine- and leucine-rich protein. Northern blot hybridisation with a specific cDNA probe revealed differential levels of expression of 6.5 kb transcripts in 35 human tissues and cancer cell lines, with strongest signals observed in fetal brain and kidney; adult testis, ovary, brain and heart; and in the SW480, K-562, HeLa S3 and HL-60 cell lines. To reflect this feature, the novel gene was designated DENN for differentially expressed in normal and neoplastic cells. Furthermore, an alternative splicing event within DENN involving a 129 nt alternative exon encoding 43 aa was found in fetal liver and in several human cancer cell lines. Based on the predicted aa sequence, two peptides were designed and synthesised to raise rabbit polyclonal antisera which detected a distinct protein band of 140-145 kDa in Western blots of human cell lines. Immunofluorescent labelling of human cells with the same antibodies indicated predominant cell membrane localisation with some cytoplasmic staining.

PMID: 8988362 [PubMed - indexed for MEDLINE]

1: Eur J Hum Genet. 1996;4(3):127-34. Related Articles, Links

Analysis of alternative splicing patterns in the cystic fibrosis transmembrane conductance regulator gene using mRNA derived from lymphoblastoid cells of cystic fibrosis patients.

Bienvenu T, Beldjord C, Chelly J, Fonknechten N, Hubert D, Dusser D, Kaplan JC.

Laboratoire de Biochimie Genetique, CHU Cochin, Paris, France.

Using in vitro amplification of cDNA by the polymerase chain reaction, we analyzed alternatively spliced events of cystic fibrosis transmembrane conductance regulator gene in lymphoblastoid cells. Ten alternatively spliced transcripts were identified using analysis of 6 overlapping segments of amplified cDNA, 4 of which have not been described previously. These include transcripts lacking exon 16, 17b, 22 and a transcript resulting from the use of a cryptic acceptor and donor splice sites. Moreover, in 2 cystic fibrosis (CF) patients bearing nonsense mutations E60X or W1282X, we observed that nonsense mutations are associated with an alteration of splice site selection in vivo resulting in exon skipping of constitutive exons or in the use of cryptic splice sites. In addition, even though lymphoblastoid cells are not the relevant tissue to address the question of the relationship between clinical respiratory phenotype and genotype, our results concerning adult CF patients (delta F508/ delta F508) suggest that individual-specific RNA splicing patterns could influence the severity of the CF pulmonary disease. If this phenomenon of alternative splicing events proves to be significant in CF and to be a common feature of disease genes, the study of RNA splicing could become an important tool for the analysis of the genotype-phenotype relationship in many inherited disorders.

PMID: 8840112 [PubMed - indexed for MEDLINE]

1: Hum Mutat. 1996;7(3):219-27. Related Articles, Links

Splice site mutation causing deletion of exon 21 sequences from the pro alpha 2(I) chain of type I collagen in a patient with severe dentinogenesis imperfecta but very mild osteogenesis imperfecta.

Nicholls AC, Oliver J, McCarron S, Winter GB, Pope FM.

Dermatology Research Group, Clinical Research Centre, Harrow, UK.

An eight-year-old boy was referred for dental assessment of dentinogenesis imperfecta, a full clinical examination also revealed joint hypermobility and some features of mild osteogenesis imperfecta although he had suffered few fractures. Analysis of the collagens produced by both gingival and skin fibroblast cultures showed the synthesis and intracellular retention of an abnormal alpha 2(I) chain that migrated faster than normal on SDS-PAGE. Cyanogen bromide peptide mapping of this intracellular protein indicated a probable deletion in the N-terminal peptide alpha 2CB4. The denaturation temperature of the mutant protein was only 36 degrees C, some 6 degrees C below normal. At 37 degrees C secretion of abnormal protein was not detectable but a lower temperature (30 degrees C) some was secreted into the medium. RT-PCR amplification of mRNA coding for alpha 2CB4 revealed a heterozygous deletion of the 108 bp exon 21 of COL1A2. Sequencing of PCR amplified genomic DNA identified a G --> A transition in the moderately conserved + 5 position of the IVS 21 5' consensus splice site causing the skipping of exon 21. Hybridization with allele-specific oligonucleotides showed no other family member had this base change. Since the cDNA deletion was associated with the (-) allele of a Pvu II polymorphism in exon 25 of COL1A2 we could demonstrate that the mutant pre-mRNA was alternatively spliced yielding both full length and deleted transcripts. Family genotype analysis indicated the mutation had originated in the paternal alpha 2(I) gene.

Publication Types:
- Case Reports

PMID: 8829655 [PubMed - indexed for MEDLINE]

1: Gene. 1995 Dec 12;166(2):339-40.

Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

A novel NF-kappa B p65 spliced transcript lacking exons 6 and 7 in a non-small cell lung carcinoma cell line.

Maxwell SA, Mukhopadhyay T.

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Transcripts of the gene encoding the p65 subunit of the NF-kappa B/Rel transcription factor complex have been reported to undergo alternative splicing to generate one derivative lacking codons for amino acids (aa) 222 to 231 (p65 delta 1) and another that lacks codons for aa 13 to 25 (p65 delta 2) of the conserved Rel homology domain [Narayaran et al., Science 256 (1992) 317-320; Lyle et al., Gene 138 (1994) 265-266]. We have identified a third splicing event in a non-small-cell lung carcinoma cell line that potentially generates a novel p65 mRNA derivative lacking codons for aa 187 to 293 (p65 delta 3) of the Rel homology domain.

PMID: 8543190 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 1995 Oct 24;92(22):10322-6.

Nonsense mutation in the phosphofructokinase muscle subunit gene associated with retention of intron 10 in one of the isolated transcripts in Ashkenazi Jewish patients with Tarui disease.

Vasconcelos O, Sivakumar K, Dalakas MC, Quezado M, Nagle J, Leon-Monzon M, Dubnick M, Gajdusek DC, Goldfarb LG.

Clinical Neurogenetics Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA.

Mutations in the human phosphofructokinase muscle subunit gene (PFKM) are known to cause myopathy classified as glycogenosis type VII (Tarui disease). Previously described molecular defects include base substitutions altering encoded amino acids or resulting in abnormal splicing. We report a mutation resulting in phosphofructokinase deficiency in three patients from an Ashkenazi Jewish family. Using a reverse transcription PCR assay, PFKM subunit transcripts differing by length were detected in skeletal muscle tissue of all three affected subjects. In the longer transcript, an insertion of 252 nucleotides totally homologous to the structure of the 10th intron of the PFKM gene was found separating exon 10 from exon 11. In addition, two single base transitions were identified by direct sequencing: [exon 6; codon 95; CGA (Arg) to TGA (stop)] and [exon 7; codon 172; ACC (Thr) to ACT (Thr)] in either transcript. Single-stranded conformational polymorphism and restriction enzyme analyses confirmed the presence of these point substitutions in genomic DNA and strongly suggested homozygosity for the pathogenic allele. The nonsense mutation at codon 95 appeared solely responsible for the phenotype in these patients, further expanding genetic heterogeneity of Tarui disease. Transcripts with and without intron 10 arising from identical mutant alleles probably resulted from differential pre-mRNA processing and may represent a novel message from the PFKM gene.

Publication Types:
- Case Reports

PMID: 7479776 [PubMed - indexed for MEDLINE]

1: Biochem Biophys Res Commun. 1995 Oct 13;215(2):713-20. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Retinoic acid induces three newly cloned HOXA1 transcripts in MCF7 breast cancer cells.

Chariot A, Moreau L, Senterre G, Sobel ME, Castronovo V.

Metastasis Research Laboratory, University of Liege, Belgium.

Coordinated expression of genes involved in development, differentiation and malignant transformation is regulated by transcription factors including homeodomain-containing proteins. However, most of their cDNA sequences are still unknown. We report here the molecular characterization of three newly cloned HOXA1 transcripts from human breast cancer cells. In addition, we provide evidence that these alternatively spliced transcripts encode one homeodomain-containing protein and two products lacking the conserved DNA-binding domain. Moreover, we demonstrate that all three HOXA1 transcripts are induced by retinoic acid in MCF7 cells. Taken together, our results suggest that HOXA1 gene may be a key element in the establishment of the breast cancer cell phenotype.

PMID: 7488013 [PubMed - indexed for MEDLINE]

1: J Neurosci Res. 1995 Oct 1;42(2):159-71.

Related Articles, Links

Differentially expressed genes after peripheral nerve injury.

Gillen C, Gleichmann M, Spreyer P, Muller HW.

Department of Neurology, University of Dusseldorf, Germany.

In an attempt to identify genes associated with Wallerian degeneration and peripheral nerve regeneration we have performed differential hybridization screening of a cDNA library from crushed rat sciatic nerve (7 days postlesion) using radioactively labeled cDNA prepared from poly (A)+ RNA of normal vs. crushed nerve. Screening of 5,000 randomly selected colonies yielded 24 distinct clones that were regulated following nerve injury. Fifteen of the differentially expressed sequences could be classified as induced, whereas 9 sequences appeared to be repressed at 1 week postcrush. Sequencing and computer-assisted sequence comparison revealed 3 classes of regulated cDNA clones representing 1) novel gene sequences (8 clones) including 3 transcripts containing a repetitive "brain identifier" (ID) element; 2) identified genes (7 clones) with previously undetected expression in the peripheral nervous system (PNS), such as apolipoprotein D, peripheral myelin protein 22kD (PMP22), SPARC (secreted protein, acidic and rich in cysteine), sulfated glycoprotein SGP-1, apoferritin, decorin, and X16/SRp20; and 3) identified genes (9 clones) with known expression in the PNS including, e.g., the myelin protein P0, gamma-actin, vimentin, alpha-tubulin, chargerin II, and cytochrome c-oxidase subunit I. Northern blot and polymerase chain reaction analyses with RNA from crushed and transected nerve demonstrated that sequences with related function, like the group of myelin genes, cytoskeleton genes, genes involved in RNA processing and translation, in lipid transport or energy metabolism showed closely related temporal patterns of expression during nerve degeneration and regeneration. Finally, we compared the differentially expressed genes identified at 7 days after crush injury (this investigation) with the regulated sequences isolated previously by De Leon et al. (J Neurosci Res 29:437-488, 1991) from a 3 day postcrush sciatic nerve cDNA library.

PMID: 8568916 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab. 1995 Oct;80(10):2933-9.

Related Articles, Links

Identification of alternatively spliced messenger ribonucleic acid encoding truncated growth hormone-releasing hormone receptor in human pituitary adenomas.

Hashimoto K, Koga M, Motomura T, Kasayama S, Kouhara H, Ohnishi T, Arita N, Hayakawa T, Sato B, Kishimoto T.

Department of Medicine III, Osaka University Medical School, Japan.

The expression of GHRH receptor (GHRH-R) messenger ribonucleic acid (mRNA) was studied in 22 pituitary adenomas and 2 normal anterior pituitaries. Northern blot analysis revealed that GHRH-R mRNA were expressed in all 14 GH-producing adenomas, 1 of 3 ACTH-producing adenomas, the 1 PRL-producing adenoma, 2 of 4 nonfunctioning adenomas, and the 2 normal anterior pituitaries. Their expression levels varied among GH-producing adenomas and were relatively low in GH-nonproducing adenomas. In addition to the major transcript with a molecular mass of 2.0 kilobases (kb), the transcripts were identified at 2.8 and 4.5 kb in some GH-producing adenomas. To examine the structural variations in GHRH-R mRNA in pituitary adenomas, we amplified the complementary DNA fragment encompassing the region from the third cytoplasmic loop to the sixth transmembrane domain of GHRH-R. This region was selected because this region of the G protein-coupled receptor has been known to interact with G protein. Two amplified fragments with the molecular masses of 250 and 810 base pairs were identified by the reverse transcriptase-polymerase chain reaction method. The nucleotide sequence of a smaller fragment, which was the expected size, revealed that no mutations were found in this region in 10 GH-producing adenomas examined. However, a larger fragment contained the currently unidentified insertion. Compared with the genomic DNA sequence, this insertion was found to be generated through alternative splicing. In addition, this variant form contained the premature stop codon in-frame, indicating that it encodes the truncated GHRH-R. This insertion-specific probe could hybridize with 2.8- and 4.5-kb species of GHRH-R mRNA on Northern blot analysis, and these transcripts were expressed mainly in GH-producing adenomas. Finally, study of cell transfection and cAMP measurement revealed that this truncated GHRH-R was unable to transmit GHRH signals. These results suggest that some GH-producing adenomas preferentially express the truncated GHRH-R as a nonfunctioning receptor through alternative splicing.

PMID: 7559877 [PubMed - indexed for MEDLINE]

1: Br J Cancer. 1995 Sep;72(3):702-7.

Related Articles, Links

Alternative promoter usage and mRNA splicing pathways for parathyroid hormone-related protein in normal tissues and tumours.

Southby J, O'Keeffe LM, Martin TJ, Gillespie MT.

St. Vincent's Institute of Medical Research, St. Vincent's Hospital, Victoria, Australia.

The parathyroid hormone-related protein (PTHrP) gene consists of nine exons and allows the production of multiple PTHrP mRNA species via the use of three promoters and 5' and 3' alternative splicing; as a result of 3' alternative splicing one of three protein isoforms may be produced. This organisation has potential for tissue-specific splicing patterns. We examined PTHrP mRNA expression and splicing patterns in a series of tumours and normal tissues, using the sensitive reverse transcription-polymerase chain reaction (RT-PCR) technique. Use of promoter 3 and mRNA specifying the 141 amino acid PTHrP isoform were detected in all samples. Transcripts encoding the 139 amino acid isoform were detected in all but two samples. Use of promoters 1 and 2 was less widespread as was detection of mRNA encoding the 173 amino acid isoform. While different PTHrP splicing patterns were observed between tumours, no tissue- or tumour-specific transcripts were detected. In comparing normal and tumour tissue from the same patient, an increase in the number of promoters utilised was observed in the tumour tissue. Furthermore, mRNA for the PTH/PTHrP receptor was detected in all samples, thus the PTHrP produced by these tumours may potentially act in an autocrine or paracrine fashion.

PMID: 7669584 [PubMed - indexed for MEDLINE]

1: Int J Cancer. 1995 Aug 22;64(4):234-8.

Related Articles, Links

Analysis of the GAP-related domain of the neurofibromatosis type 1 (NF1) gene in childhood brain tumors.

Scheurlen WG, Senf L.

Department of Pediatrics, University of Wurzburg, Germany.

The neurofibromatosis type-1 (NF1) gene contains a 360-bp region with significant homology to the catalytic domain of mammalian GTPase-activating protein. This particular GAP-related domain of the NF1 gene (NF1-GRD) stimulates ras GTPase and inactivates ras protein p21ras. Therefore, it has been suggested that the NF1 gene represents another tumor-suppressor gene. In the search for molecular markers of possible diagnostic relevance, childhood brain-tumor specimens of different histologic diagnoses were tested for mutations of the so-called FLR-exon within the NF1-GRD. This part of the NF1-GRD has been shown to be most crucial for the GAP-like function. Using a highly sensitive PCR-SSCP technique, we tested 51 tumor specimens were tested, but found no mutations. We conclude that inactivation of this putative tumor-suppressor gene by mutations does not play a significant role in tumorigenesis of childhood brain tumors. Next, we compared the splice variants of the NF1-GRD in 33 brain tumors and 8 extraneural embryonal tumors. Primitive neuroectodermal tumors (PNET) (n = 10) and one intracranial teratoma were the only tumors that predominantly expressed a splice pattern that can be observed in the immature developing brain. In contrast to other embryonal neuronal tumors, this NF1-GRD splicing pattern could not be modified in a newly established medulloblastoma cell line by retinoic acid treatment. Since this particular splice variant suppresses p21ras more effectively than other NF1-GRD transcripts, its predominant expression may interfere with the physiological signal transduction of p21ras during differentiation of neurons. There may be a neurofibromin-induced and p21ras-mediated differentiation pathway of neuronal stem cells that is blocked in PNET. Such an arrest of a p21ras-dependent differentiation pathway may explain the persistence of primitive pluripotent neuronal cells in PNET.

PMID: 7657385 [PubMed - indexed for MEDLINE]

1: Eur J Biochem. 1996 Sep 15;240(3):732-7.

Related Articles, Links

Alternative processing of the tryptophanyl-tRNA synthetase mRNA from interferon-treated human cells.

Turpaev KT, Zakhariev VM, Sokolova IV, Narovlyansky AN, Amchenkova AM, Justesen J, Frolova LY.

Engelhardt Institute of Molecular Biology, Moscow, Russia.

We have analysed the structure of mRNA isoforms of the human gene encoding tryptophanyl-tRNA synthetase (Trp-tRNA synthetase) expressed in the epithelial CaOv cells and MT-4 lymphocytes. The Trp-tRNA synthetase gene is induced by interferon-gamma in both lines and, in MT-4 lymphocytes, also by interferon-alpha. Four Trp-tRNA synthetase mRNA isoforms have different combinations of the first exons IA, IB and II. Two transcription initiation sites (P1 and P2) were detected 90 bp from each other. Processing of the primary transcript initiated from the P1 start site generates the mRNA isoform where exon IA joins to exon II. The other three isoforms are produced by alternative splicing of the primary transcript produced from the P2 start site. Isoform 2 has a 3'-end fragment of exon IA joined to exon II. Isoform 3 contains exons IA and IB. Isoform 4 contains exon IA and exon III and lacks exon II encoding the N-terminus of the Trp-tRNA synthetase. Therefore, the two primary transcripts of the Trp-tRNA synthetase gene differ only in the 5' flank sequence between P1 and P2, and this fragment regulates their processing. Both interferon-alpha and interferon-gamma induce exon IA-containing and exon IB-containing isoforms of the Trp-tRNA synthetase mRNA.

PMID: 8856077 [PubMed - indexed for MEDLINE]

1: Mol Endocrinol. 1995 Aug;9(8):959-68. Related Articles, Links

Functionally different isoforms of the human calcitonin receptor result from alternative splicing of the gene transcript.

Moore EE, Kuestner RE, Stroop SD, Grant FJ, Matthewes SL, Brady CL, Sexton PM, Findlay DM.

ZymoGenetics, Seattle, Washington 98102, USA.

Two subtypes of the human calcitonin receptor (hCTR) have been described which differ from one another by the presence or absence of a 16-amino acid insert in the first intracellular loop. Both isoforms were stably expressed in baby hamster kidney cells to compare their ligand binding and second messenger coupling. The binding affinity and the on/off rate of binding for salmon CT were identical for the two receptor isoforms. However, the presence of the insert significantly reduced the ability of the receptor to couple to both adenylate cyclase and phospholipase C. Stimulation of a transient calcium response was only observed with the insert-negative receptor. Similarly, the ED50 for the cAMP response is 100-fold higher for the insert-positive form compared with the insert-negative form of the receptor. However, the maximal cAMP response was equivalent for both receptor isoforms. The rate of internalization of the insert-positive form of the receptor is significantly impaired relative to the insert-negative receptor, which suggests that this process may be dependent on the stimulation of a second messenger pathway. Cloning and characterization of the relevant portion of the hCTR gene revealed that these isoforms are generated by alternative splicing. We also discovered a third isoform of the hCTR, which can be generated by alternative splicing at the same position. The presence of a stop codon in this newly described alternative exon would lead to premature termination of the receptor at the C-terminal end of the first transmembrane domain.

PMID: 7476993 [PubMed - indexed for MEDLINE]

1: Oncogene. 1995 Apr 6;10(7):1377-83. Related Articles, Links

Multiple mRNA isoforms of the human RET proto-oncogene generated by alternate splicing.

Lorenzo MJ, Eng C, Mulligan LM, Stonehouse TJ, Healey CS, Ponder BA, Smith DP.

Department of Pathology, University of Cambridge, UK.

The RET proto-oncogene encodes a receptor tyrosine kinase. We and others have recently shown that distinct germline mutations of the RET proto-oncogene account for the majority of cases of the dominantly inherited multiple endocrine neoplasia (MEN) type 2 syndromes, and can cause a dominantly inherited form of Hirschsprung disease, a disorder of development of the autonomic innervation of the gut. RET is also oncogenically activated in some sporadic thyroid and adrenal tumours. Here we report the characterisation of multiple mRNA isoforms of RET generated by alternate splicing. Two isoforms are predicted to encode membrane-spanning receptors with a truncated extracellular ligand-binding domain. A third isoform is predicted to encode a soluble, secreted form of the receptor. These mRNA isoforms are expressed in both normal and tumour tissues.

PMID: 7731689 [PubMed - indexed for MEDLINE]

1: Biochem Biophys Res Commun. 1995 Aug 4;213(1):342-8. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Survival motor neuron gene transcript analysis in muscles from spinal muscular atrophy patients.

Gennarelli M, Lucarelli M, Capon F, Pizzuti A, Merlini L, Angelini C, Novelli G, Dallapiccola B.

Dipartimento di Sanita Pubblica e Biologia Cellulare, Universita Tor Vergata, Roma, Italy.

We have identified and characterized four different mRNA isoforms of the survival motor neuron (SMN) gene from skeletal muscle of 9 SMA patients and 15 unaffected controls. These isoforms appear to be generated by combinatorial splicing of both exons 5 and 7 of the SMN telomeric and centromeric gene copies. The full-size and the truncated SMN-1b isoforms of the telomeric SMN copy are significantly reduced in muscle of SMA patients, irrespective of the disease types. Our results suggest that multiple RNA splicing is operative in the two SMN-related genes and that SMN-related polypeptides may be active in the muscle.

PMID: 7639755 [PubMed - indexed for MEDLINE]

1: Nucleic Acids Res. 1995 Jul 25;23(14):2762-9. Related Articles, Links

Alternative splicing and genomic structure of the AML1 gene involved in acute myeloid leukemia.

Miyoshi H, Ohira M, Shimizu K, Mitani K, Hirai H, Imai T, Yokoyama K, Soeda E, Ohki M.

Radiobiology Division National Cancer Center Research Institute, Tokyo, Japan.

We previously isolated the AML1 gene, which is rearranged by the t(8;21) translocation in acute myeloid leukemia. The AML1 gene is highly homologous to the Drosophila segmentation gene runt and the mouse transcription factor PEBP2 alpha subunit gene. This region of homology, called the Runt domain, is responsible for DNA-binding and protein--protein interaction. In this study, we isolated and characterized various forms of AML1 cDNAs which reflect a complex pattern of mRNA species. Analysis of these cDNAs has led to the identification of two distinct AML1 proteins, designated AML1b (453 amino acids) and AML1c (480 amino acids), which differ markedly from the previously reported AML1a (250 amino acids) with regard to their C-terminal regions, although all three contain the Runt domain. The large C-terminal region common to AML1b and AML1c is suggested to be a transcriptional activation domain. AML1c differs from AML1b by only 32 amino acids in the N-terminal. Characterization of the genomic structure revealed that the AML1 gene consists of nine exons and spans > 150 kb of genomic DNA. Northern blot analysis demonstrated the presence of six major transcripts, encoding AML1b or AML1c, which can all be explained by the existence of two promoters, alternative splicing and differential usage of three polyadenylation sites. A minor transcript encoding AML1a which results from alternative splicing of a separate exon can be detected only by reverse transcription-polymerase chain reaction amplification. The distinct proteins encoded by the AML1 gene may have different functions, which could contribute to regulating cell growth and/or differentiation through transcriptional regulation of a specific subset of target genes.

PMID: 7651838 [PubMed - indexed for MEDLINE]

1: Neurosci Lett. 1995 Jul 28;195(1):1-4. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Altered expression of dopamine D2 receptor mRNA splice variants in brain and pituitary of spontaneously hypertensive rats.

Autelitano DJ, van den Buuse M.

Molecular Physiology Laboratory, Baker Medical Research Institute, Prahran, Victoria, Australia.

Both central and peripheral dopamine (DA) has been shown to play a role in the regulation of blood pressure. Using sensitive nuclease protection analysis, we have compared the expression of DA D-2 receptor (D2-R) mRNA splice variants in brain and pituitary of spontaneously hypertensive rats (SHR) with normotensive Wistar-Kyoto (WKY) controls. Levels of D2-R mRNA were significantly altered in pituitary anterior lobe (AL) and neurointermediate lobe (NIL), and in striatum of SHR, but not in any other brain regions examined. SHR pituitary expressed 50-80% higher levels of D2-R mRNA, coupled with an increase in the relative proportion of the long (D2-L) mRNA variant. In contrast, overall D2-R mRNA expression in SHR striatum was only 75% that of WKY controls, however, the relative proportion of the D2-L splice variant was increased. The present data demonstrate that tissue specific alterations in D2-R mRNA levels and primary transcript splicing exist in the SHR and suggest that these changes may in part mediate differential responsiveness to DA that may be related to the development of hypertension.

PMID: 7478242 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 1995 Jul 18;92(15):7041-5.

A splice variant of alpha 6 integrin is associated with malignant conversion in mouse skin tumorigenesis.

Tennenbaum T, Belanger AJ, Glick AB, Tamura R, Quaranta V, Yuspa SH.

Laboratory of Cellular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

The epithelial-specific integrin alpha 6 beta 4 is suprabasally expressed in benign skin tumors (papillomas) and is diffusely expressed in carcinomas associated with an increase in the proliferating compartment. Analysis of RNA samples by reverse transcriptase-PCR and DNA sequencing revealed that chemically or oncogenically induced papillomas (n = 8) expressed a single transcript of the alpha 6 subunit, identified as the alpha 6 A splice variant. In contrast, carcinomas (n = 13) expressed both alpha 6A and an alternatively spliced form, alpha 6B. Primary keratinocytes and a number of keratinocyte cell lines that vary in biological potential from normal skin, to benign papillomas, to well-differentiated slowly growing carcinomas exclusively expressed alpha 6A. However, I7, an oncogene-induced cell line that produces highly invasive carcinomas, expressed both alpha 6A and alpha 6B transcript and protein. The expression of alpha 6B in I7 cells was associated with increased attachment to a laminin matrix compared to cell lines exclusively expressing alpha 6A. Furthermore, introduction of an alpha 6B expression vector into a papilloma cell line expressing alpha 6A increased laminin attachment. When a papilloma cell line was converted to an invasive carcinoma by introduction of the v-fos oncogene, the malignant cells expressed both alpha 6A and alpha 6B, while the parent cell line and cells transduced with v-jun or c-myc, which retained the papilloma phenotype, expressed only alpha 6A. Comparative analysis of alpha 6B expression in cell lines and their derived tumors indicate that alpha 6B transcripts are more abundant in tumors than cell lines, and alpha 6B is expressed to a greater extent in poorly differentiated tumors. These results establish a link between malignant conversion and invasion of squamous tumor cells and the regulation of transcript processing of the alpha 6 beta 4 integrin.

PMID: 7624366 [PubMed - indexed for MEDLINE]

1: Blood. 1995 Jul 1;86(1):277-82.

Related Articles, Links

RT-PCR diagnosis of patients with acute nonlymphocytic leukemia and inv(16) (p13q22) and identification of new alternative splicing in CBFB-MYH11 transcripts.

van der Reijden BA, Lombardo M, Dauwerse HG, Giles RH, Muhlematter D, Bellomo MJ, Wessels HW, Beverstock GC, van Ommen GJ, Hagemeijer A, et al.

Department of Human Genetics, Leiden University, Sylvius Laboratories, The Netherlands.

As acute nonlymphocytic leukemia (ANLL) with inv(16) (p13q22) or t(16;16)(p13;q22) has been shown to result from the fusion of transcription factor subunit core binding factor (CBFB) to a myosin heavy chain (MYH11), we sought to design methods to detect this rearrangement using reverse transcriptase-polymerase chain reaction (RT-PCR). In all of 27 inv(16)(p13q22) and four t (16;16)(p13;q22) cases tested, a chimeric CBFB-MYH11 transcript coding for an in-frame fusion protein was detected. In a more extensive RT-PCR analysis with different primer pairs, we detected a second new chimeric CBFB-MYH11 transcript in 10 of 11 patients tested. The CBFB-MYH11 reading frame of the second transcript was maintained in one patient but not in the others. We show that the different CBFB-MYH11 transcripts in one patient arise from alternative splicing. Translation of the transcript in which the CBFB-MYH11 reading frame is not maintained leads to a slightly truncated CBFB protein.

PMID: 7795233 [PubMed - indexed for MEDLINE]

1: Oncogene. 1995 Jun 15;10(12):2331-42. Related Articles, Links

The proto-oncogene FGF-3 is constitutively expressed in tumorigenic, but not in non-tumorigenic, clones of a human colon carcinoma cell line.

Galdemard C, Brison O, Lavialle C.

Laboratoire de Genetique Oncologique, URA 1967 CNRS, Institut Gustave Roussy, Villejuif, France.

The human colon carcinoma cell line, SW613-S, is composed of cells with a high-level amplification of the MYC proto-oncogene that are tumorigenic in nude mice and of cells with a low-level amplification of MYC that are not tumorigenic. Transcripts from FGF-3, a member of the fibroblast growth factor gene family, accumulate in cells from tumorigenic clones, but are undetectable in those from non-tumorigenic clones. Nuclear run-on analyses indicate that this differential FGF-3 expression is regulated at the level of transcription initiation. Determination of the structure of the FGF-3 transcripts indicates that they are generated by splicing of the three exons and termination at the single polyadenylation site predicted from the genomic sequence. Their size heterogeneity is due to multiple initiation sites spanning a 700 base-pair long promoter region. FGF-3 is activated in tumors induced in nude mice by MYC-transfected cells from non-tumorigenic clones. However, in most of the cell lines established from these tumors, FGF-3 expression tends to be lost upon in vitro propagation. Thus, in these transfectant cell lines, the presence of exogenous MYC gene copies is not sufficient to activate FGF-3 expression and in vivo growth is also required.

PMID: 7784081 [PubMed - indexed for MEDLINE]

1: Cancer Res. 1995 Jun 15;55(12):2542-7. Related Articles, Links

An alternatively spliced form of NQO1 (DT-diaphorase) messenger RNA lacking the putative quinone substrate binding site is present in human normal and tumor tissues.

Gasdaska PY, Fisher H, Powis G.

Arizona Cancer Center, University of Arizona, Tucson 85724, USA.

DT-diaphorase is a ubiquitously expressed flavoenzyme responsible for the two-electron reduction of a number of quinone and other anticancer drugs. The majority of DT-diaphorase enzyme activity in human tissues is the product of the NQO1 gene. We have now identified a novel alternatively spliced form of human NQO1 mRNA lacking exon 4 at levels equal to or exceeding those of wild-type NQO1 mRNA. Exon 4 codes for the putative quinone substrate binding site of DT-diaphorase derived from NQO1 and the recombinant protein from alternatively spliced NQO1 mRNA lacking exon 4 has minimal enzyme activity with quinoid and other known substrates of DT-diaphorase. The physiological substrate of DT-diaphorase is unknown, and it is possible that the protein derived from the alternatively spliced NQO1 mRNA could have enzyme activity with an appropriate substrate. We found full-length DT-diaphorase protein but could not detect expression of an appropriately smaller form of DT-diaphorase in human tissues using polyclonal antibody to DT-diaphorase, suggesting that alternatively spliced NQO1 mRNA lacking exon 4 may not be translated or that the protein product is rapidly degraded. Alternative splicing of NQO1 RNA could provide an important mechanism for regulating NQO1 gene expression.

PMID: 7780966 [PubMed - indexed for MEDLINE]

1: Genomics. 1995 Jun 10;27(3):475-80. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Alternative splicing of the tuberous sclerosis 2 (TSC2) gene in human and mouse tissues.

Xu L, Sterner C, Maheshwar MM, Wilson PJ, Nellist M, Short PM, Haines JL, Sampson JR, Ramesh V.

Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown 02129, USA.

The recently isolated gene for tuberous sclerosis 2 (TSC2) encodes a 5.5-kb transcript that is widely expressed. The TSC2 gene product, named tuberin, is a 1784-amino-acid protein that shows a small stretch of homology to the GTPase activating protein rap1GAP. We have detected a novel variant of the TSC2 mRNA lacking 129 nucleotides, predicting an in-frame deletion of 43 amino acids spanning codons 946-988 of tuberin. This 129-bp deletion precisely corresponds to exon 25 of the TSC2 gene suggesting that alternative splicing leads to production of two forms of transcripts designated isoforms 1 and 2. Further molecular analysis revealed a third isoform exhibiting a deletion of 44 amino acids spanning codons 946-989 of tuberin. Amino acid 989 is a Ser residue encoded by the first codon of exon 26. The two isoforms also exist in newborn and adult mouse tissues, reinforcing the potential functional importance of these alternatively spliced products. These alternative isoforms should have implications for efforts aimed at identifying mutations in TSC patients. The distinct polypeptides encoded by the TSC2 gene may have different targets as well as functions involved in the regulation of cell growth.

PMID: 7558029 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1995 Jun 2;270(22):13326-32. Related Articles, Links

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Regulation of alternative splicing of protein kinase C beta by insulin.

Chalfant CE, Mischak H, Watson JE, Winkler BC, Goodnight J, Farese RV, Cooper DR.

Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa, USA.

Insulin regulates a diverse array of cellular signaling processes involved in the control of growth, differentiation, and cellular metabolism. Insulin increases glucose transport via a protein kinase C (PKC)-dependent pathway in BC3H-1 myocytes, but the function of specific PKC isozymes in insulin action has not been elucidated. Two isoforms of PKC beta result via alternative splicing of precursor mRNA. As now shown, both isoforms are present in BC3H-1 myocytes, and insulin induces alternative splicing of the PKC beta mRNA thereby switching expression from PKC beta I to PKC beta II mRNA. This effect occurs rapidly (15 min after insulin treatment) and is dose-dependent. The switch in mRNA is reflected by increases in the protein levels of PKC beta II. High levels of 12-0-tetradecanoylphorbol-13-acetate, which are commonly used to deplete or down-regulate PKC in cells, also induce the switch to PKC beta II mRNA following overnight treatment, and protein levels of PKC beta II reflected mRNA increases. To investigate the functional importance of the shift in PKC beta isoform expression, stable transfectants of NIH-3T3 fibroblasts overexpressing PKC beta I and PKC beta II were established. The overexpression of PKC beta II but not PKC beta I in NIH-3T3 cells significantly enhanced insulin effects on glucose transport. This suggests that PKC beta II may be more selective than PKC beta I for enhancing the glucose transport effects of insulin in at least certain cells and, furthermore, that insulin can regulate the expression of PKC beta II by alternative mRNA splicing.

PMID: 7768933 [PubMed - indexed for MEDLINE]

1: J Lipid Res. 1995 Jun;36(6):1315-24. Related Articles, Links

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Occurrence of multiple aberrantly spliced mRNAs of the LDL-receptor gene upon a donor splice site mutation that causes familial hypercholesterolemia (FHBenevento).

Lelli N, Garuti R, Ghisellini M, Tiozzo R, Rolleri M, Aimale V, Ginocchio E, Naselli A, Bertolini S, Calandra S.

Dipartimento di Scienze Biomediche, Universita di Modena, Italy.

A novel point mutation of the LDL-receptor gene was found in an Italian patient with homozygous familial hypercholesterolemia. The SSCP analysis of the promoter and of 16 out of the 18 exons of the LDL-receptor gene was negative, suggesting that the mutation might be located in the region of the gene encompassing exons 14 and 15, a region that had not been amenable to polymerase chain reaction (PCR) amplification from genomic DNA. This region was amplified from cDNA by reverse transcription PCR (RT-PCR). RT-PCR of proband cDNA generated three fragments of 800, 600, and 550 bp, respectively, as opposed to a single 720 bp fragment obtained from control cDNA. The sequence of these fragments showed that: i) in the 800-bp fragment exon 14 continued with the 5' end of intron 15 (90 nucleotides), which in turn was followed by exon 16; ii) in the 600-bp fragment exon 14 was followed by the 5' end of exon 15 (50 nucleotides), which continued with exon 16; iii) in the 550-bp fragment exon 14 joined directly to exon 16. These abnormally spliced mRNAs resulted from a G-->A transition at the +1 nucleotide of intron 15, which changed the invariant GT dinucleotide of the 5' donor splice site. That was associated with the activation of two cryptic donor splice sites in intron 15 and exon 15, respectively, and the use of an alternative splicing leading to the skipping of exon 15. Northern blot analysis showed that the overall content of these aberrantly spliced mRNAs in proband fibroblasts was one-fourth that found in control cells. These abnormally spliced mRNAs are predicted to encode three abnormal receptor proteins: the first would contain an insertion of 30 novel amino acids; the second would be a truncated protein of 709 amino acids; the third would be devoid of the 57 amino acids of the O-linked sugar domain. Ligand blot experiments indicated that the amount of LDL-receptor present in proband's fibroblasts was approximately one-tenth that found in control cells.

Publication Types:
- Case Reports

PMID: 7545204 [PubMed - indexed for MEDLINE]

1: Exp Eye Res. 1995 Apr;60(4):401-6. Related Articles, Links

Alternative splicing in human retinal mRNA transcripts of an opsin-related protein.

Jiang M, Shen D, Tao L, Pandey S, Heller K, Fong HK.

Department of Microbiology, University of Southern California School of Medicine, Los Angeles 90033, USA.

An opsin-related gene encodes a putative RPE-retinal G-protein-coupled receptor (RGR) that is most homologous to the visual pigments and invertebrate retinochrome. A splice variant of human RGR mRNA can be demonstrated by the sequence of isolated cDNA clones and by the amplification and analysis of human retinal mRNA. The shortened transcript contains a deletion of 114 nucleotides that correspond exactly to the sequence of exon 6 in the human rgr gene. The predicted RGR variant lacks the putative sixth transmembrane domain and has a calculated molecular weight of 27,726. Variable amounts of a 28-kDa protein were found in the retinas of some individuals by immunoblot assay. Since a similar shortened RGR transcript was not detected in bovine retina or RPE, the RGR variant is not essential for vertebrate vision. Analysis of the structure of the rgr gene and of the sequences of cDNA clones indicates that the truncated mRNA may be produced through alternative splicing of pre-mRNA from which a cassette exon is removed and the predicted RGR variant is radically altered in primary structure.

PMID: 7789419 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab. 1995 Apr;80(4):1247-52. Related Articles, Links

Screening for growth hormone (GH) gene splice-site mutations in sporadic cases with severe isolated GH deficiency using ectopic transcript analysis.

Binder G, Ranke MB.

University Children's Hospital, Tubingen, Germany.

We screened 10 children with sporadic severe isolated GH deficiency (IGHD) for GH-1 gene splice site mutations using ectopic transcript analysis. None had a history of birth trauma, congenital defects, thyroid disorders, or PRL deficiency. The mean age of these patients at diagnosis was 3.5 yr; the mean height at diagnosis was -4.0 SD score. GH-1 gene deletion was excluded in all cases. Ribonucleic acid (RNA) from lymphocytes was reverse transcribed and amplified by nested polymerase chain reaction, using two primer pairs with annealing sites within exons 2 and 5 of the GH-1 gene. The main polymerase chain reaction fragment obtained was 460 basepairs and proved to be the amplification of the GH-1 transcript. We also found three shorter fragments which were alternatively spliced GH-1 transcripts, including a variant devoid of the first 45 basepairs of exon 3, a second lacking the whole exon 3, and a third one, not previously described, lacking both exon 3 and exon 4. We found the same pattern of alternative splicing in RNA from GH-producing pituitary tumor tissue, which served as a positive control. In 1 of 10 patients, a pathologically shortened main fragment lacking exon 3 was detected. As proved by sequencing genomic DNA, this was the result of a heterozygous splice site mutation, with transversion from G to C of the first base of the donor splice site of intron III generating a new DdeI recognition site. The other allele had no mutation. DdeI digestion enabled us to rule out the defect in the parents' DNA. Thus, the mutation was de novo. As the patient with the mutation displayed the most severe and earliest growth retardation in the study group and had virtually no GH in serum, it must be assumed that the heterozygous genetic defect resulted in a dominant negative effect. The reason for this is still unclear. Recently, within a family that exhibited the autosomal dominant phenotype of IGHD (IGHD-II), a heterozygous point mutation was located 5 bases down-stream from that we describe here. A similar effect on splicing was observed. In conclusion, analysis of ectopic GH-1 transcripts enabled us to detect 1) a new alternatively spliced GH-1 messenger RNA variant lacking exons 3 and 4, and 2) 1 of 10 sporadic cases of severe idiopathic IGHD due to a heterozygous de novo splice site mutation in the GH-1 gene that changes G to C in the first base of intron III.(ABSTRACT TRUNCATED AT 400 WORDS)

PMID: 7714096 [PubMed - indexed for MEDLINE]

1: Hum Genet. 1995 Apr;95(4):391-6. Related Articles, Links

Human ferrochelatase: a novel mutation in patients with erythropoietic protoporphyria and an isoform caused by alternative splicing.

Schneider-Yin X, Schafer BW, Tonz O, Minder EI.

Zentrallabor, Stadtspital Triemli, Zurich, Switzerland.

Erythropoietic protoporphyria (EPP), attributable to deficiency of ferrochelatase activity (FECH), is characterised mainly by cutaneous photosensitivity. To define the molecular defect in two EPP-affected siblings and their parents in a Swiss family, ferrochelatase cDNA was amplified by the polymerase chain reaction (PCR) and subjected to sequence analysis. A 5-bp deletion (T580-G584) was identified on one allele of the ferrochelatase gene in both patients and their mother. Screening of the mutation among family members of RsaI digestion of PCR-amplified genomic DNA revealed autosomal dominant inheritance associated with abnormal protoporphyrin concentration and enzyme activity. We also isolated ferrochelatase cDNAs containing a 18-bp insertion (part of the intron 2 sequence) between exons 2 and 3; this corresponded to six extra amino acids (YESNIR) inserted between Arg-65 and Lys-66 of the known ferrochelatase. This isoform was identified initially in mRNAs derived from both alleles of the ferrochelatase gene in one patient. Its existence was confirmed in six additional EPP patients, in five out of seven controls, and in four different cell lines (fibroblast, muscle, hepatoma and myelogenous leukaemia). This isoform, roughly 20% of the total ferrochelatase mRNA, was generated through splicing at a second donor site in intron 2 and its presence was not linked to EPP.

Publication Types:
- Case Reports

PMID: 7705834 [PubMed - indexed for MEDLINE]

1: Mol Cell Endocrinol. 1995 Apr 1;109(2):197-207.

Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Differential expression of estrogen receptor mRNA splice variants in the tamoxifen resistant human breast cancer cell line, MCF-7/TAMR-1 compared to the parental MCF-7 cell line.

Madsen MW, Reiter BE, Lykkesfeldt AE.

Department of Tumor Endocrinology, Danish Cancer Society, Copenhagen.

Breast cancer patients with an estrogen receptor (ER) positive tumor can be treated with the anti-estrogen tamoxifen, but development of anti-estrogen resistance is a serious problem. We have analyzed a tamoxifen resistant human breast cancer cell line MCF-7/TAMR-1 for alterations in ER which might explain the tamoxifen resistance. The MCF-7/TAMR-1 cells expressed both wild-type ER mRNA and protein, and by RT-PCR we were able to clone ER cDNAs corresponding to the following mRNA splice variants: ER delta E2, ER delta E4, ER delta E5, ER delta E7 and a new double splice variant lacking both exon 4 and 7 (ER delta E4,7) The existence of the ER delta E4,7 variant was confirmed by RNase protection assay. Semi-quantitative RT-PCR revealed that ER delta E2 mRNA was expressed at a higher level in MCF-7/TAMR-1 cells, whereas the ER delta E5 mRNA was expressed at a significantly lower level in MCF-7/TAMR-1 cells compared with MCF-7 cells. The differential expression of the two ER mRNA splice variants indicates that they may be involved in anti-estrogen resistance, although the present knowledge of their biological function does not provide us with an explanation.

PMID: 7664983 [PubMed - indexed for MEDLINE]

1: J Mol Cell Cardiol. 1995 Apr;27(4):981-90. Related Articles, Links

Fibronectin expression during physiological and pathological cardiac growth.

Farhadian F, Contard F, Corbier A, Barrieux A, Rappaport L, Samuel JL.

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Fibronectin (FN) is a dimeric glycoprotein found in the extracellular matrix of most tissues that serves as a bridge between cells and the interstitial collagen meshwork and influences diverse processes including cell growth, adhesion, migration, and wound repair. Multiple FN forms arise by the alternative splicing of a primary transcript originating from a single gene. The spatial and temporal alterations in FN expression in the myocardium has been studied in models of cardiac growth in vivo such as fetal development, and hypertrophy secondary to pressure overload. This review focuses on the differential expression of FN isoforms that are observed in different models of cardiac growth. Using a combination of qualitative and quantitative analyses it is shown that in the rat myocardium: (1) the FN phenotype is developmentally regulated, (2) the re-expression of the fetal FN isoforms is observed in different models of cardiac hypertrophy secondary to a sudden or progressive hypertension and (3) the changes in cardiac FN expression affect mostly the coronary artery smooth muscle cells.

Publication Types:
- Review
- Review, Tutorial

PMID: 7563110 [PubMed - indexed for MEDLINE]

1: Ann N Y Acad Sci. 1995 Mar 27;752:470-91. Related Articles, Links

X-linked dilated cardiomyopathy. Novel mutation of the dystrophin gene.

Franz WM, Cremer M, Herrmann R, Grunig E, Fogel W, Scheffold T, Goebel HH, Kircheisen R, Kubler W, Voit T, et al.

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We report on a family with a severe form of X-linked dilated cardiomyopathy (DCM). Two brothers, the elder requiring heart transplantation, and a maternal cousin presented elevated creatine kinase levels, increased right ventricular diameters and electrocardiographic abnormalities. All complained of exertional cramping myalgia, but none had muscle weakness or a pathological electromyogram. Muscle biopsies of these individuals revealed a mild myopathic picture with atrophic type I and hypertrophic type II fibers. Immunofluorescence using N- and C-terminal antibodies (dys-2, dys-3) against the dystrophin protein showed preserved, but reduced intensity of staining of the sarcolemmal membranes. Using the same two antibodies, Western blot analyses revealed a dystrophin molecule of the expected molecular weight, which was quantitatively reduced by 80%. However, the dys-1 antibody, directed against the mid rod region of the dystrophin protein, did not react with dystrophin both on Western blot and immunofluorescence. Linkage analysis with polymorphic markers of the dystrophin gene revealed an identical haplotype at the 5' region in all affected individuals (two point lod score of 1.93, phi = 0). A deletion of exons 48, 45-53, 2-7 and 1 including the promoter region of the dystrophin gene, as described in rare cases with similar clinical signs could be excluded by multiplex PCR and Southern blot analyses of this DCM family. In addition, a major splice-mutation of dystrophin mRNA was excluded by RT-PCR of skeletal and heart muscle tissue. Therefore, we conclude that a novel mutation in the 5' region of the dystrophin gene phenotypically leads to this severe form of DCM. Extensive analyses of the dystrophin gene, in particular of the sequences coding for the antigenic determinants of the dys-1 antibody in the mid rod region, may identify the molecular cause of this monogenetic form of DCM.

Publication Types:
- Case Reports

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1: Cancer Res. 1995 Mar 15;55(6):1261-6. Related Articles, Links

Comment in:
- Cancer Res. 1995 Sep 1;55(17):3933-4.

Malignant and nonmalignant brain tissues differ in their messenger RNA expression patterns for ERCC1 and ERCC2.

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Perturbation of the DNA repair process appears to be responsible for the occurrence of a number of human diseases, which are usually associated with a propensity to develop internal malignancies and/or disorders of the central nervous system. We have been interested in the possibility that a subtle abnormality in DNA repair competency might be associated with the transformation of nonmalignant cells to the malignant state. To study this question, we assayed malignant and nonmalignant brain tissues from 19 individuals for mRNA expression levels of the human DNA repair genes ERCC1, ERCC2, and XPAC and for differential splicing of the ERCC1 transcript. We separately compared expression levels of these genes in the following situations: concordance of expression within malignant tissues; concordance of expression within nonmalignant tissues; concordance between malignant and nonmalignant tissues within individuals of the cohort; and concordance of gene expression between two nonmalignant tissue sites within a single individual. Linear regression analyses of mRNA values obtained suggested orderly concordance of these three DNA repair genes in nonmalignant tissues within the patient cohort and an excellent concordance of these genes between two separate biopsy sites from the same individual. In contrast, malignant tissues showed disruption of concordance between the full-length ERCC1 transcript and ERCC2, which have excision and helicase functions, respectively. Furthermore, within the same individuals, malignant tissues were discordant with nonmalignant tissues for ERCC1 and ERCC2, although concordance for XPAC was preserved. These data suggest that one molecular characteristic of human malignancy may be the disruption of the normal relationship between the excision and the helicase functions of the nucleotide excision repair pathway.

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